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FIELD RESPONSE OF RED-SPANISH PINEAPPLES TO NITROGEN, CALCIUM, IRON AND SOIL pH

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[1] As of date of issue, November, 1958
[2] Leave to pursue studies
[3] Other leaves

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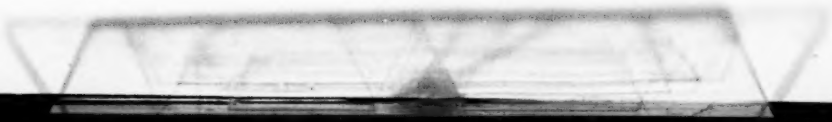

FIELD RESPONSE OF RED-SPANISH PINEAPPLES TO NITROGEN, CALCIUM, IRON AND SOIL pH

WILLIAM PENNOCK [1]

INTRODUCTION

In accordance with Hopkin's [2] findings with respect to the effect of iron and manganese on pineapples, three field experiments [3] were started in 1945 by T. G. Mason, Plant Physiologist of this Station at that time. These experiments were later placed in charged of E. Hernández, Assistant Physiologist, and were turned over to the author in January 1948. The primary objective of these experiments was to determine if pineapple yields could be increased by the use of lime amendments calculated to raise soil pH to 6.5. At this pH much of the available manganese, which is excessive for pineapples in some Puerto Rican soils, would be expected to be immobilized while much of the available iron would still remain available. Since spraying with ferrous sulfate is commonly used as a supplementary source of available iron, and fertilizing with ammonium sulfate tends to lower soil pH, the experiment was designed to include two levels of ferrous sulfate spraying and two levels of nitrogen source fertilizing. Since gypsum was not available because of the war, the use of rock phosphate in combination with lime carbonate was also incorporated in the experiment as a source of additional calcium which would not be expected to alter soil pH markedly.

The two experiments, located in Arecibo and Río Piedras, respectively, had similar layouts consisting of 132 plots each $40' \times 9'$ or A./121. Each plot had 60 plants at $20'' \times 20''$ spacing, planted on the 3 rows per bank system. One acre included 7,260 plants. Twelve treatments were replicated eleven times in randomized blocks. In the Río Piedras experiment, however, one replication was discarded because mealy bug in-

[1] Associate Horticulturist, Agricultural Experiment Station, Río Piedras, P. R.

[2] Hopkins, E. F., Pagán V., and Ramirez Silva, F. J.; Iron and Manganese in Relation to Plant Growth and its Importance in Puerto Rico. Journ. Agr. U.P.R. XXVIII No. 2, Apr. 1944.

[3] One experiment located in Corozal was discontinued after the first harvest data were taken because the fruit size was quite small, having a mean weight of 2.03 lbs. The results showed no significant effect from any of the treatments except spray. The results were omitted from this discussion because they were of no particular value and yet involved some complicating differences in the experimental set up.

festation affected the yields adversely without regard to treatment. In this experiment, therefore, the pertinent data comprise only 120 plots.

A factorial design involving three levels of calcium supplements, two levels of ferrous sulfate spraying and two levels of nitrogen source was employed. The three levels of calcium consisted of the untreated control, calcium carbonate at a rate calculated to bring up soil pH to 6.5 and calcium carbonate at the same rate plus rock phosphate at 1 ton per acre. In Arecibo the calcium carbonate was applied at the rate of 4 tons per acre; in Río Piedras at 7 tons per acre.

The two levels of ferrous sulfate spraying consisted of the Puerto Rican schedule and the Hawaiian schedule, respectively. The Puerto Rican schedule consisted of a single spraying at 8 months from planting. The Hawaiian schedule consisted of 17 sprayings; semimonthly during the first 3 months followed by monthly spraying until bloom emergence occurred at roughly 13 months from planting. The spray consisted of 25 lbs. of ferrous sulfate per 100 gals. of water applied at about 30 gals. per acre.

The two nitrogen source levels consisted of nitrogen supplied as ammonium sulfate and ammonium nitrate. In both cases it was applied at the rate of 390 lbs. of NH_3 per acre distributed as follows:

20 per cent on planting
30 per cent after 5 months
30 per cent after 10 months
20 per cent after 15 months

Potash and phosphorous were mixed with the nitrogen fertilizer and applied at a total rate per acre of 290 lbs. K_2O and 170 lbs. of P_2O_5. The fertilizer constituents were mixed together and applied directly to the lower leaves of the individual plants.

The Río Piedras experiment was planted on July 23–28, 1945; the Arecibo experiment on August 3, 1945. Both were acetylene treated in September 1946 to provoke blooming and were harvested in February 1947. The Arecibo experiment was on Bayamón silty clay and the Río Piedras experiment on Fa-

jardo clay. The Arecibo soil had previously been cropped almost continuously to pineapples, during the past fifteen years whereas, the Río Piedras soil had been in pasture for about the same length of time. Soil samples for pH determination were taken from both experiments in October 1947; 8 months after the plantilla harvests.

At Arecibo each plot was sampled at two soil levels; approximately 0–2″ and 2–6″ depth, respectively. At Río Piedras each plot was sampled at three levels: 0″–2″, 2″–4″ and 4″–6″ depth, respectively. Three borings were made for each plot of both experiments and aggregate samples made for each plot with soil taken from the same level in the 3 borings. The borings were located in the center and at 10 ft. from both extremities of each plot, and therefore in every case were located midway between adjacent plants. None of the borings were made at the immediate base of the individual plants where greatest fertilizer accumulation occurred.

The pH determinations were made by the Soils Department with a quinhydrone electrode. Iron and manganese determinations are not yet available.

The plantilla or first crop was harvested in February 1946. The fruit was weighed and counted separately for each plot. Data were also taken with reference to the percentage incidence of gum disease of the fruit and the production of "slips" or axillary offshoots from the fruiting stalk, which are used as planting material. For this publication we have decided to limit discussion to mean fruit weight yields. Table 1 shows the mean fruit weights obtained for the mean of each of the treatments given.

TABLE No. 1—PINEAPPLE YIELDS OBTAINED IN PLANTILLA HARVESTS AT ARECIBO AND RIO PIEDRAS, FEB. 1946

Symbol	Treatments Given			Treatment Means	
				Arecibo	Río Piedras
	Calcium	Source of N	Type of Spray	Mean Weight of Fruit	Mean Weight of Fruit
				lbs.	lbs.
A^1	None	Am. Sulfate	Puerto Rico	3.84	4.00
A^2	None	Am. Sulfate	Hawaii	4.13	3.98
A^3	None	Am. Nitrate	Puerto Rico	3.69	3.56
A^4	None	Am. Nitrate	Hawaii	3.94	3.77
B^1	Lime alone	Am. Sulfate	Puerto Rico	4.12	3.44
B^2	Lime alone	Am. Sulfate	Hawaii	4.05	3.66
B^3	Lime alone	Am. Nitrate	Puerto Rico	3.82	3.00
B^4	Lime alone	Am. Nitrate	Hawaii	4.12	3.13
C^1	Lime Rock phosphate.	Am. Sulfate	Puerto Rico	3.88	3.27
C^2	Lime Rock phosphate.	Am. Sulfate	Hawaii	4.13	3.47
C^3	Lime Rock phosphate.	Am. Nitrate	Puerto Rico	3.59	3.02
C^4	Lime Rock phosphate.	Am. Nitrate	Hawaii	3.83	2.86
General Mean				3.93	3.43
Minimum difference for 5% sign				.29	.425
Minimum difference for 1% sign				.39	.563
F values for treatments				3.13	6.37

The mean fruit weight data for each experiment were first subjected to analysis of variance without regard to soil pH. A summary of these analyses are shown in tables 2 and 3 for Arecibo and Río Piedras, respectively.

TABLE No. 2—ARECIBO EXPERIMENT — PLANTILLA CROP

A summary of analysis of variance showing the partition of treatment sums of squares of dev ations of the mean fruit weights in pounds per fruit into main effects and interactions. The corresponding variance and "F" values are also shown.

Source	D. F.	S. S.	Variance	F.
Main Factors				
Calcium	2	.6880	.3440	2.89
Sprays	1	1.4721	1.4721	12.35**
Nitrogen	1	1.2335	1.2335	10.35**
First order Interactions:				
Calcium×Sprays	2	.1480	.0740	.62
Calcium×Nitrogen	2	.1856	.0928	.78
Spray×Nitrogen	1	.0855	.0855	.71
Second order Interaction:				
Calcium×Sprays×Nitrogen	2	.2898	.1449	1.22
Total Treatments	11	4.1025		
Error	110	13.1129	.1192	
Replications	10	4.2014		
TOTAL	131	21.4168		

TABLE NO. 3—RIO PIEDRAS EXPERIMENT — PLANTILLA CROP

A summary of analysis of variance showing the partition of treatment sums of squares of deviations of the mean fruit weights in pounds per fruit into main effects and interactions. The corresponding variances and "F" values are also shown.

Source Main Factors	D. F.	S. S.	Variance	F.
Calcium	2	9.9651	4.9825	21.71**
Sprays	1	.2736	.2736	1.19
Nitrogen	1	5.1461	5.1461	22.42**
First Order Interactions				
Calcium×Sprays	2	.1179	.0589	.26
Calcium×Nitrogen	2	.1326	.0664	.29
Spray-Nitrogen	1	.0323	.0323	.14
Second Order Interaction				
Calcium×Sprays×Nitrogen	2	.4148	.2074	.90
Total Treatments	11	16.0824	.....	
Error	99	22.7172	.2295	
Replications	9	17.8008	.....	
TOTAL	119	56.6002	.....	

Thereafter, the pH data for each level of soil from both experiments were grouped in accordance with pH range and then plotted against mean weight of fruit. Figures 1–5 show the curves thus obtained. Analyses by rank correlation showed that the pH of the soil at 0–2″ depth in Río Piedras, with a Rho value of .475, had the highest correlation with yields of all the sets of data pertaining to soil pH in both experiments. Since as may be seen in figure 3, the relationship very closely approximates a straight line, the tentative assumption was made, therefore, that yield response in pineapples normally bears a linear or nearly linear relationship to soil pH within the experimental range. Departures from this linear relationship observed in figures 1–5 were presumed to have been caused by other factors affecting yield. A further corollary assumption was made that, if the yield response of numerous plants to soil pH was linear, the yield response of a single plant, whose roots were embedded in a mosaic of soil having varying pH, was also probably linear or nearly so and not logarithmic.

On this assumption a mean soil pH for the top 6 inches of soil was calculated for each experiment using the pH indexes directly without conversion to the antilogarithms. In the Río Piedras experiment, where 3 pH determinations were made of three successive levels at even intervals of two inches, the mean soil pH was obtained from the arithmetic mean of the three figures for each plot. In the Arecibo experiment, where the soil

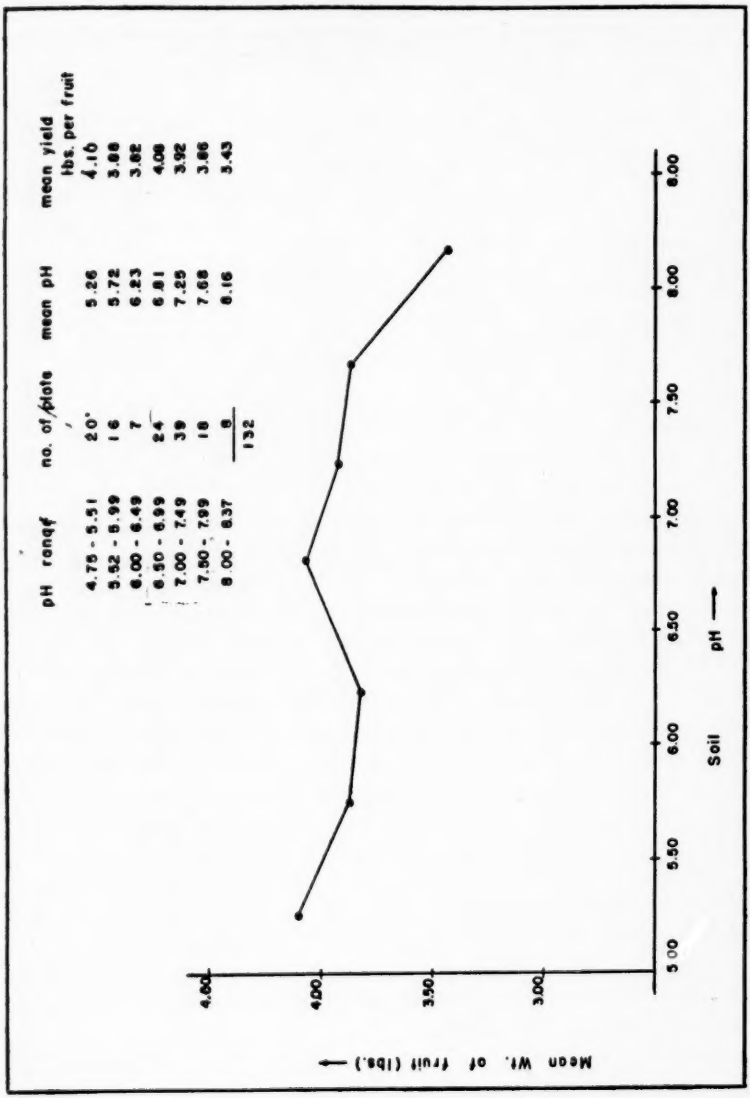

FIG. 1.—ARECIBO EXPERIMENT: Soil pH at 0–2" depth plotted against mean fruit weight.

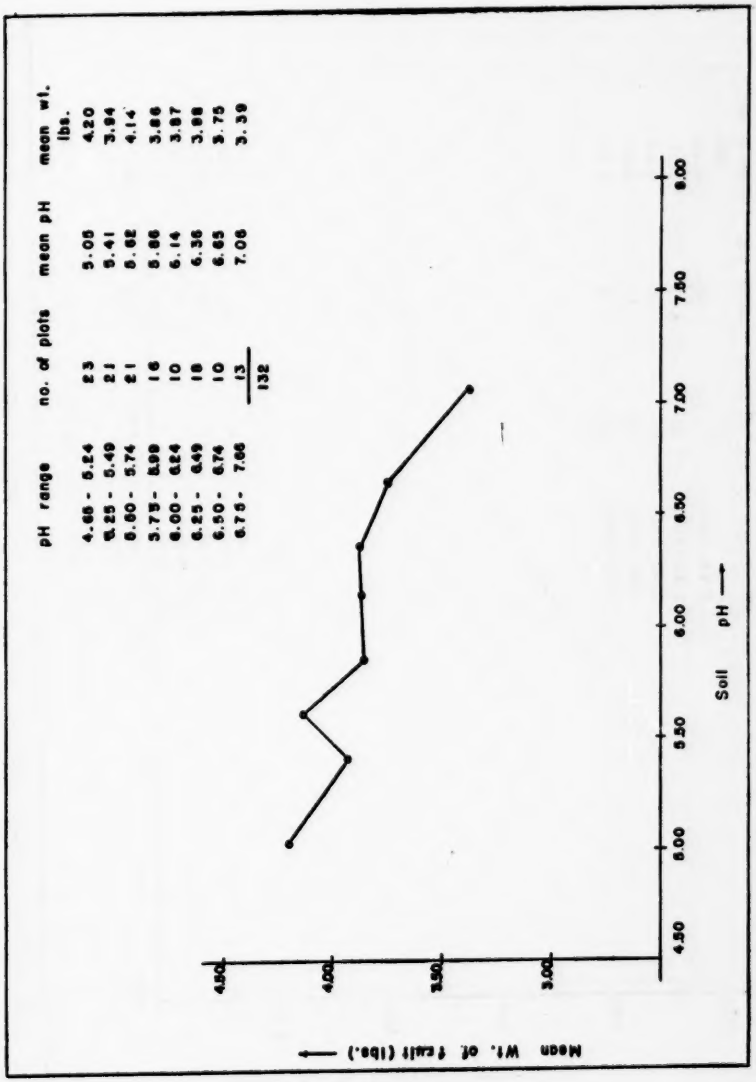

FIG. 2.—ARECIBO EXPERIMENT: Soil pH at 2'-6" depth plotted against mean fruit weight.

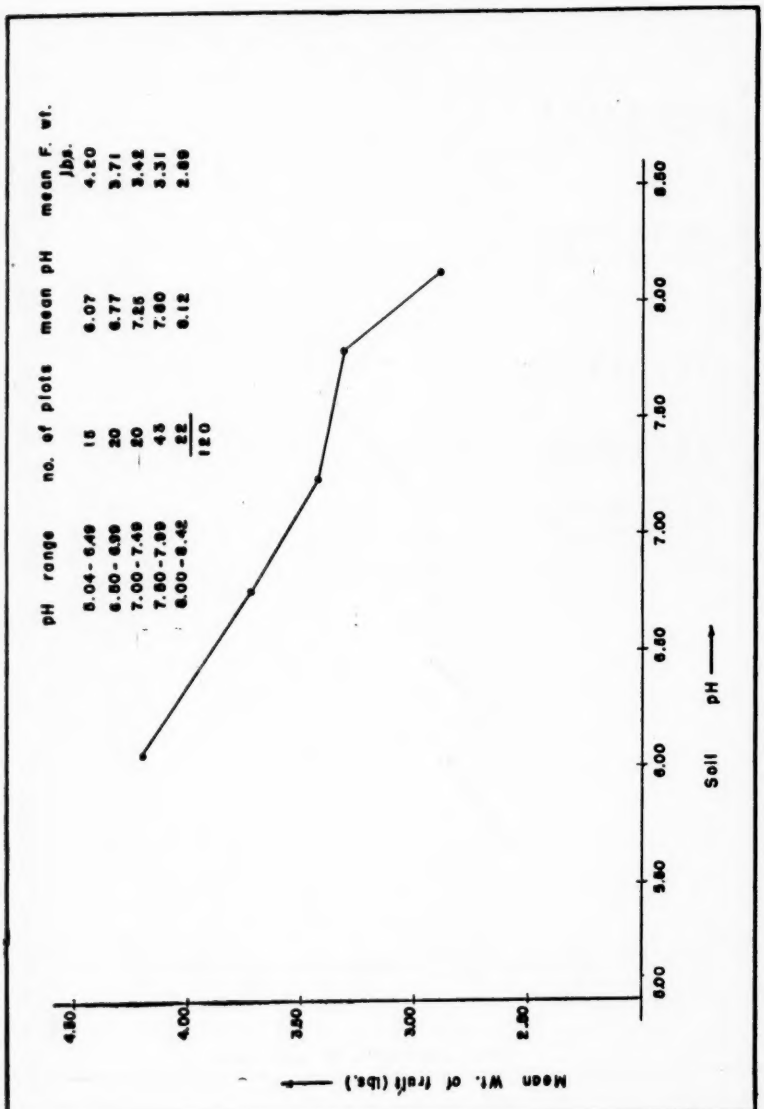

FIG. 3.—RIO PIEDRAS EXPERIMENT: Soil pH at 0–2" depth plotted against mean fruit weight.

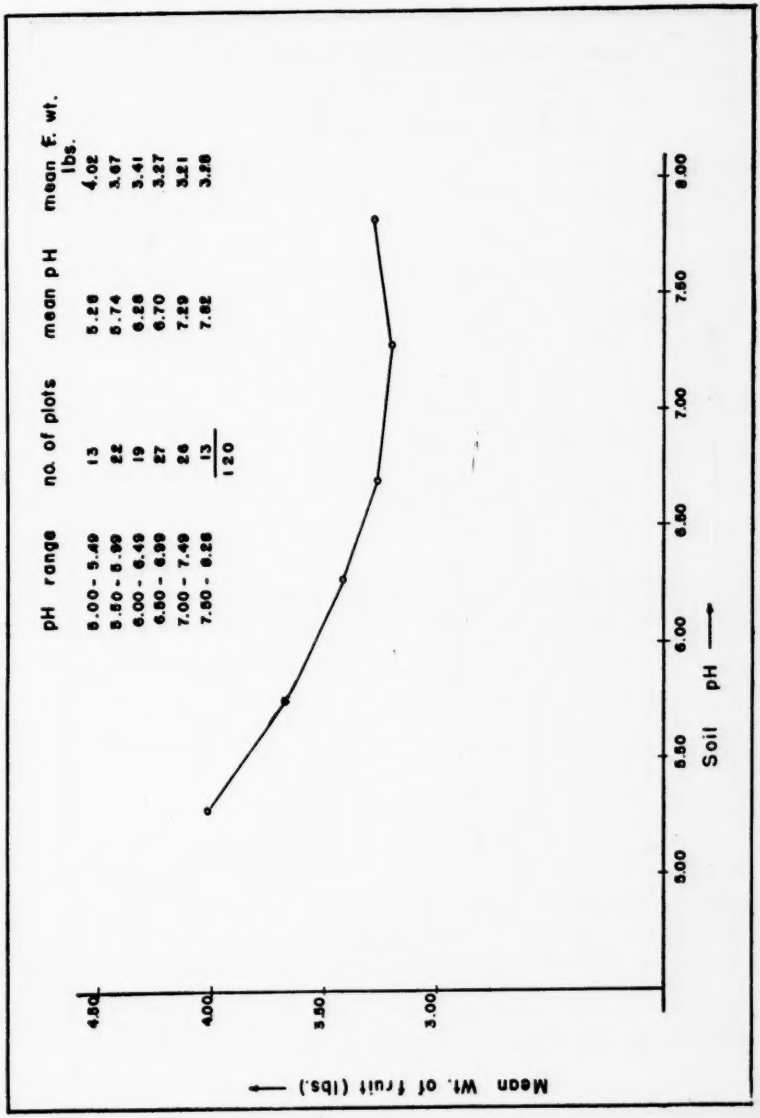

FIG. 4.—RIO PIEDRAS EXPERIMENT: Soil pH at 2'-4" plotted against mean fruit weight.

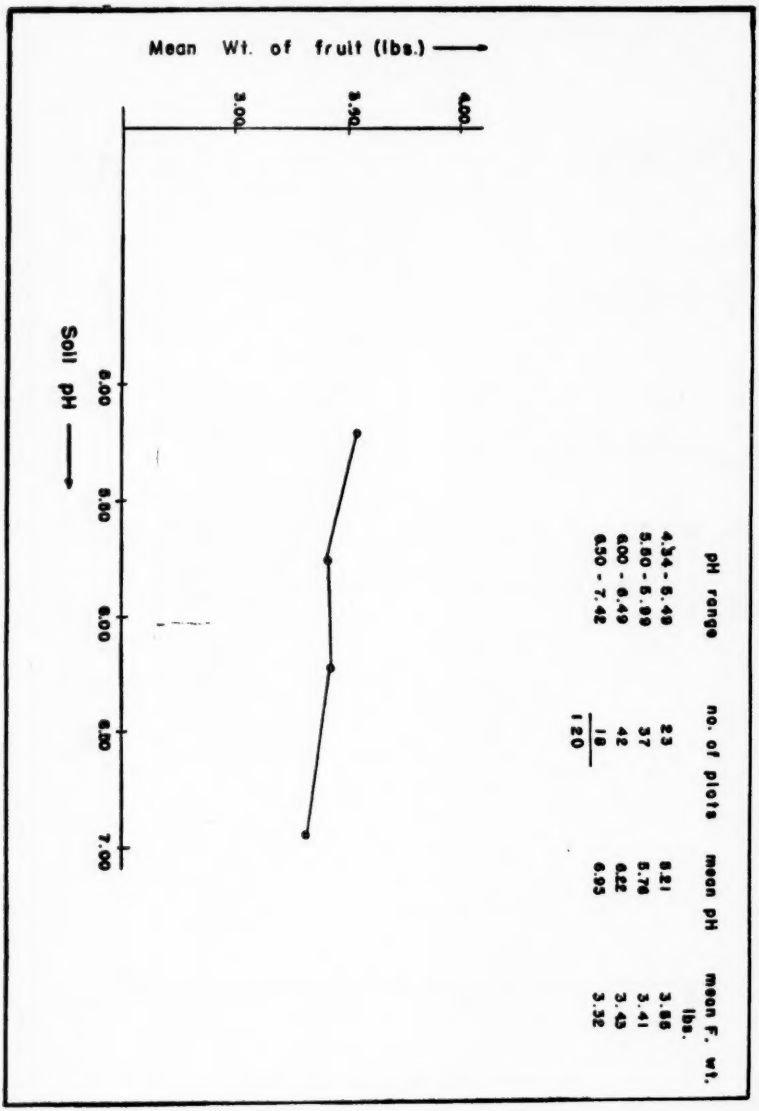

FIG. 5.—RIO PIEDRAS EXPERIMENT: Soil pH at 4'-6" depth plotted against mean fruit weight.

was sampled at 0–2″ and 2″–6″ levels, the lower level figures were given double weight for calculating the mean for each plot. The mean soil pH indexes thus obtained were grouped by pH intervals and plotted against mean fruit weight as shown in figures 6 and 7.

The effect of treatments on mean soil pH was then determined by analysis of variance, a summary of which is shown in tables 4 and 5.

TABLE NO. 4—Summary of analysis of variance showing the effect of treatments on mean soil pH in Arecibo experiment

Source	D. F.	S. S.	Variance	F.
Calcium	2	4.8109	2.4055	10.996**
Sprays	1	.0433	.0433	.....
Nitrogen	1	.0130	.0130	.....
Interactions	7	2.0675	.2954	1.350
Replications	10	11.1724	1.1172	.....
Error	110	24.0630	.2188	.....
TOTAL	131	42.1701		

F value for 2 and 110 D. F. at 1% = 4.80.
F value for 7 and 110 D. F. at 5% = 2.10.

TABLE NO. 5—Summary of analysis of variance showing the effect of treatments on mean soil pH in Río Piedras Experiment.

Source	D. F.	S. S.	Variance	F.
Calcium	2	13.8537	6.9269	85.715**
Sprays	1	.0337	.0337	.....
Nitrogen	1	.0859	.0859	.....
Interactions	7	1.2274	.1753	2.170*
Error	108	8.7278	.0808	
TOTAL	119	28.5118		

F. value for 2 and 108 D. F. at 1% = 4.80.
F. value for 7 and 108 D. F. at 5% = 2.10.
F. value for 7 and 108 D. F. at 1% = 2.82.

As may be seen in tables 4 and 5, the nitrogen source and spray treatments did not affect soil pH and can, therefore, be discussed conveniently before explaining further steps in the manipulation of the data.

THE EFFECT OF NITROGEN SOURCE ON MEAN FRUIT WEIGHT

As may be seen in tables 2 and 3, differences in nitrogen source treatments affected mean fruit weight by amounts which were highly significant in both experiments and had no significant interaction with any of the other treatments. Table 6 shows the comparisons with regard to nitrogen treatments in both experiments. As may be seen in table 6, ammonium sul-

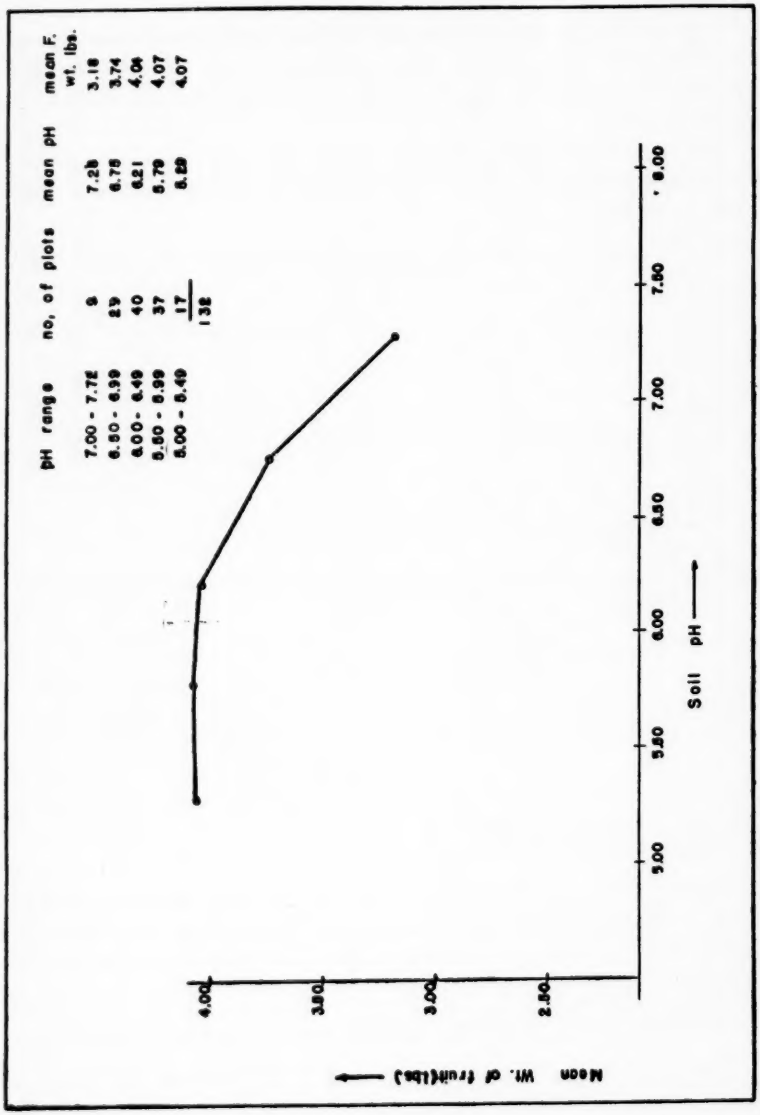

FIG. 6.—**ARECIBO EXPERIMENT:** Mean pH for top 6" of soil plotted against mean fruit weight.

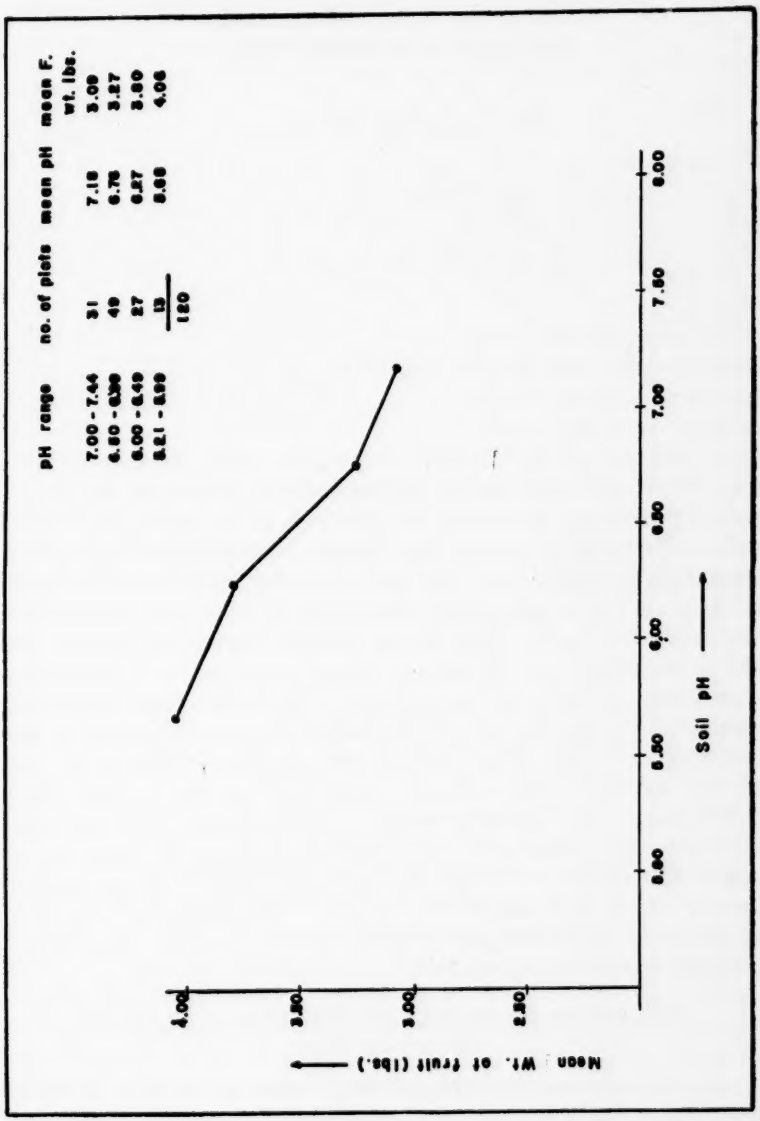

FIG. 7.—RIO PIEDRAS EXPERIMENT: Mean pH for top 6" of soil plotted against mean fruit weight.

TABLE No. 6—Comparison of treatments in Arecibo and Río Piedras with respect to response in plantilla yields to nitrogen source treatments.

AMMONIUM SULFATE VS. AMMONIUM NITRATE

Symbols	Conditions Governing Comparison	Differences Between Treatment Totals	
		Arecibo	Río Piedras
A_1—A_3	No calcium, Puerto Rico spray	1.56	4.38*
A_2—A_4	No calcium, Hawaii spray	2.15	2.14
B_1—B_3	Lime alone, Puerto Rico spray	3.29*	4.52*
B_2—B_4	Lime alone, Hawaii spray	—.73	5.21*
C_1—C_3	Lime + rock phos., Puerto Rico spray	3.21*	2.54
C_2—C_4	Lime + rock phos., Hawaii spray	3.28*	6.06**
Totals		12.76	24.85
Mean difference		.193**	.414**
Sign difference between treatment totals at 5%		3.21	4.24
Sign difference between treatment totals at 1%		4.24	5.61
Sign general mean difference at 5%		.12	.17
Sign general mean difference at 1%		.16	.23

fate, when compared to ammonium nitrate, increased the mean yield by a mean difference of .193 lbs. at Arecibo, and, by a mean difference of .414 at Río Piedras. Both mean differences were highly significant. The difference in favor of ammonium sulfate, on a per acre basis, amounted to 1,400 lbs. in Arecibo and 3,000 lbs. in Río Piedras, or roughly equivalent to $42 and $90, respectively, at the cannery price of 3¢ per lb. We believe that some of this nitrogen source effect was brought about through its effect on soil pH not reflected by our method of soil sampling for pH. The fertilizer was applied in the lower leaf axil of the plant and probably leached down into the soil close to the plant. As indicated earlier, our sampling was done midway between plants and consequently could not be expected to reflect the influence of the nitrogenous fertilizer on the pH of the soil near the base of the plants. The ammonium sulfate probably lowered soil pH in these localized areas thereby increasing yields to some extent.

THE EFFECT OF FERROUS SULFATE SPRAYS ON MEAN WEIGHT OF FRUIT

As may be seen in tables 2 and 3, the differences in spray treatments had a highly significant effect in Arecibo, but a negligible effect in Río Piedras. There were no significant interactions with other treatments in either experiment. Table 7 shows the comparisons concerning spray treatments in both experiments. As may be seen in table 7 the multiple or Hawaii

TABLE NO. 7—COMPARISON SHOWING THE EFFECT OF SPRAY TREATMENTS ON PLANTILLA MEAN FRUIT WEIGHTS IN ARECIBO AND RIO PIEDRAS

HAWAII SCHEDULE VS. P. R. SCHEDULE

Symbols	Conditions Governing Cnmparison	Difference Between Treatment	
		Totals Arecibo	Totals Río Piedras
A_2—A_1	No calcium, Amm. sulfate	3.25*	—.15
A_4—A_3	No calcium, Amm. nitrate	2.66	2.09
B_2—B_1	Lime alone, Amm. sulfate	—.72	2.06
B_4—B_3	Lime alone, Amm. nitrate	3.30*	1.37
C_2—C_1	Lime + rock phos., Amm. sulfate	2.76	1.94
C_4—C_3	Lime + rock phos., Amm. nitrate	2.69	—1.58
Totals		13.94	5.73
Mean difference		.232**	.096
Significant difference between treatment totals at 5%		3.21	4.46
Significant difference between treatment totals at 1%		4.24	5.90
Significant general mean difference at 5%		.119	.174
Significant general mean difference at 1%		.158	.230

spray schedule when compared to the single Puerto Rico spray schedule, increased the mean fruit weight by a mean difference of .232 at Arecibo and .096 at Río Piedras. The mean difference was highly significant at Arecibo, but not significant at Río Piedras. The mean difference in favor of the multiple spray schedule at Arecibo, on a per acre basis, amounted to 1684 lbs. or $50 at the cannery price of 3¢ per lb. The differences are, presumably, due to increased intake of iron supplied to the plants. The experiment was not designed to show the benefits of a single spray, but it is logical to assume that to some extent it was beneficial in both experiments. One, and sometimes two sprayings of ferrous sulfate, are generally supplied by the growers and it is generally accepted by now that in most Puerto Rican soils such spraying, if applied early in the growing period, brings about increased fruit weights. The experiment shows that multiple spraying can in some fields, such as the one at Arecibo, increase fruit weight significantly over and beyond the expected increase occasioned by a single spray. These findings are in agreement with Hopkin's work with respect to iron nutrition of pineapples and stress the importance of iron nutrition as affecting yields. As shown by Hopkins and others, "iron deficiency" symptoms, which respond to ferrous sulfate spray, can be caused by excess manganese.

Before analyzing for the effect of soil pH on yields, the yield data for both experiments was first adjusted for the effect of nitrogen and sprays. The adjustment consisted in adding or subtracting one-half of the mean differences shown in tables 6

and 7. In Arecibo the nitrogen adjustment amounted to .10 lbs. which was added to ammonium nitrate plots and subtracted from ammonium sulfate plots. The spray adjustment amounted to .11 lbs. added to Puerto Rico sprayed plots and subtracted from Hawaii sprayed plots. In Río Piedras the nitrogen adjustment amounted to .21 lb. added to ammonium nitrate plots and subtracted from ammonium sulfate plots. The spray adjustment amounted to .05 lb. added to Puerto Rico sprayed plots and subtracted from Hawaii sprayed plots. Despite the lack of significance for the effect of sprays in Río Piedras, the adjustment is warranted in view of the negligible effect of sprays on soil pH shown in table 5.

With the yields thus adjusted, a partial regression analysis was made for each experiment to find the regression of yields on the following factors:

1. No calcium control
2. Lime carbonate treatment
3. Lime carbonate plus rock phosphate treatment
4. Mean soil pH
5. Mean soil pH squared

Summaries of these two analyses are found in tables 8 and 9 for Arecibo and Río Piedras, respectively.

TABLE No. 8—A SUMMARY OF PARTIAL REGRESSION ANALYSIS OF THE MEAN FRUIT WEIGHTS IN ARECIBO EXPERIMENT

Source	D. F.	S. S.	Variance	F.
Total	131	21.4168	.....	.....
Remainder after adjustment for nitrogen and spray treatments	129	18.7152	.....	.....
Remainder after adjustment for nitrogen and sprays and linear regression	126	11.7803	.0935	.....
Remainder after adjustment for nitrogen and sprays, linear regression and regression curvilinear on pH	125	11.2369	.0899	.....
Regression linear on calcium and pH	3	6.9349	2.3116	24.725
Regression linear on calcium and curvilinear on pH	4	7.4783	1.8696	20.797
Curvilinear effect of pH over and beyond its linear effect	1	.5434	.5434	6.045
Significant value at 1% level	3	.....	.....	3.94
Significant value at 1% level	4	.....	.....	3.47
Significant value at 5% level	1	.....	.....	3.92
Significant value at 1% level	1	.....	.....	6.84

Regression Coefficients	Linear pH on	T	Curvilinear on pH	T
Non-limed control	—.1279**	3.231	—.1118**	8.985
Lime carbonate treatment	.1090**	2.895	+.1004**	8.560
Lime carbonate plus rock phosphate treatment	.0189*	2.468	+.0114	.296
pH	—.4089**	8.175	1.6419	1.965
$(pH)^2$	.....	.....	—.1640**	7.778
Significant values at 5% level	.....	1.978	.....	1.979
Significant values at 1% level	.....	2.615	.....	2.616

TABLE No .9—A SUMMARY OF PARTIAL REGRESSION ANALYSIS OF THE MEAN FRUIT WEIGHTS IN RIO PIEDRAS EXPERIMENT

Source	D. F.	S. S.	Variance	F.
Total	119	56.6002	.....	.....
Remainder after adjustment for nitrogen and spray treatment	117	51.1822	.....	.....
Remainder after adjustment for nitrogen and sprays and linear regression	114	35.2666	.3094	.....
Remainder after adjustment for nitrogen and sprays and linear regression and regression curvilinear on pH	113	35.0609	.3103	.....
Regression linear on calcium and pH	3	15.9156	5.3052**	17.419
Regression linear on calcium and curvilinear on pH	4	16.1214	4.0303**	12.990
Curvilinear effect of pH over and beyond its linear effect	1	.2057	.2057	.663
Significant value at 1% level	3	.....	.....	3.96
Significant value at 1% level	4	.....	.....	3.49
Significant value at 5% level	1	.....	.....	3.93

Regression Coefficients	Linear on pH	T	Curvilinear on pH	T
Non-limed control	+.1049	.338	+.1102	1.12
Lime carbonate treatment	+.1017	1.155	+.1107	1.24
Lime carbonate plus rock phosphate treatment	—.2066	2.091*	—.2210	2.918**
pH	—.6371	4.388**	1.2273	.535
$(pH)^2$	.....	.....	—.1438	.815
Significant values at 5% level	.....	1.981	.....	1.981
Significant values at 1% level	.....	2.619	.....	2.620

THE EFFECT OF CALCIUM SUPPLEMENTS IN ARECIBO

As may be seen in table 8 the curvilinear effect of pH over and beyond its linear effect was significant. The most precise determination for the effect of calcium supplements is therefore derived from this regression in which the squares of the mean soil pH were taken into account.

For convenience in comparing the results with those obtained by the previous analysis, in which the pH effects were ignored, the mean differences due to calcium supplements, in both analyses, are shown side by side in table 10. As may be seen in

TABLE No. 10—COMPARISONS SHOWING THE EFFECT OF CALCIUM SOIL SUPPLEMENTS BEFORE AND AFTER REGRESSION CURVILINEAR FOR pH IN ARECIBO EXPERIMENT

Comparisons	Before Regression on pH[1]	After Regression Curvilinear on pH	Significant Difference	
	Mean Difference /Fruit lbs.	Mean Difference /Fruit lbs.	At 5%	At 1%
Lime carbonate vs. no calcium control	+.126	+.212**	.131	.173
Lime plus rock phosphate vs. no calcium control	—.044	+.123	.135	.179
Lime plus rock phosphate vs. lime alone	—.170*	—.089	.127	.169
Minimum significant difference at 5%	.146	.....	.....	.....
Minimum significant difference at 1%	.193	.....	.....	.....

[1] These data are derived from the original plot yields before adjustment for the effect of sprays and nitrogen. No significant interactions occurred, however, with these two factors.

table 10, the only significant difference before the curvilinear regression on pH, occurred as a result of the depressing effect on yields of rock phosphate when applied under conditions of liming. The effect of lime alone was an increase in yield which was short of a signicant value. After regression on pH, however, the lime increased yields by a mean difference of .212 lbs. per single fruit, which was highly significant and was the equivalent of 1539 lbs. or $46 per acre. The lime plus rock phosphate treatment, on the other hand, did not affect treatments significantly after regression. It is evident, therefore, that the direct effect of lime, when divorced from its indirect effect, associated with changes in soil pH, was a highly significant mean increase in yields. Presumably, the lime was beneficial as a nutrient source or by improving soil texture or by some other means not directly associated with soil pH as sampled. The deleterious effect of the rock phosphate supplement under limed conditions was, on the other hand, largely associated with the effect of mean soil pH as sampled.

THE EFFECT OF CALCIUM SUPPLEMENTS IN RIO PIEDRAS

As may be seen in table 9, the curvilinear effect of pH over and beyond its linear effect was not significant and the variance for error of the linear regression was lower than that of the regression curvilinear on pH. The most precise determination of the effect of calcium supplements is therefore derived from the linear regression. For convenience in comparing the results with those obtained by the previous analysis, in which the pH effects were ignored, the mean differences, due to calcium supplements in both analyses, are shown side by side in table 11.

TABLE No. 11—COMPARISONS SHOWING THE EFFECT OF CALCIUM SUPPLEMENTS BEFORE AND AFTER LINEAR REGRESSION FOR pH IN RIO PIEDRAS

Comparisons	Before Regression on Mean Soil pH[1]	After Linear Regression on Mean Soil pH	Significant Difference	
	Mean Difference /Fruit lbs.	Mean Difference /Fruit lbs.	At 5%	At 1%
Lime carbonate vs. no calcium control	—.520**	—.003	.340	.449
Lime plus rock phosphate vs. no calcium control	—.675**	—.311*	.296	.391
Lime plus rock phosphate vs. lime alone	—.154	—.308*	.256	.339
Minimum significant difference at 5%	.212			
Minimum significant difference ar 1%	.281			

[1] These data are derived from the original plot yields before adjustment for the effect of sprays and nitrogen. No significant interactions occurred, however, with these two factors.

As may be seen in table 11, before the regression on soil pH, the lime carbonate depressed yields by a highly significant value. The lime plus rock phosphate depressed yields to an even larger extent, but the difference attributable to the effects of rock phosphate when lime had been applied was not significant. After the linear regression on pH, however, the deleterious effect of lime was nullified almost with mathematical exactitude. The lime plus rock phosphate depressed yields significantly and this can be attributed to the harmful effects of rock phosphate under conditions of liming. Presumably lime was harmful in this experiment only because it raised mean soil pH. The harmful effect of rock phosphate, formerly not significant although appreciable, was increased to a significant value when divorced from the effect of pH by regression. In contrast with the Arecibo results, the harmful effect of rock phosphate in Río Piedras, was largely independent of the effects of soil pH.

THE EFFECT OF MEAN SOIL pH

As may be seen in table 8 in the Arecibo experiment, the linear correlation coefficient on pH was highly significant, however, the curvilinear coefficient on pH^2 also was highly significant and therefore, a second order parabola fitted the data significantly better that did a straight line.

As may be seen in table 9 in the Río Piedras experiment, the linear correlation coefficient on pH was highly significant, whereas, the curvilinear regression on pH^2 was not significant. A straight line, therefore, fitted the data significantly better than did the 2nd order parabola. However, because of theoretical considerations, we chose not to ignore entirely the best fitting parabola for this experiment.

In figures 8 and 9 are shown the best fitting straight lines and 2nd order parabolas for Arecibo and Río Piedras, respectively. The yield data for each experiment were adjusted for the effect of calcium supplements and grouped in accordance with pH range. The mean pH and mean yields of these groups were also plotted and shown in figures 8 and 9.

As may be seen in figure 8 the parabola maximum for the Arecibo experiment occurs at pH 5.00 with a mean fruit weight of 4.20 lbs. With reservations, in view of the experimental limitations, this may be considered the optimum mean soil pH and yield for Bayamón silty clay.

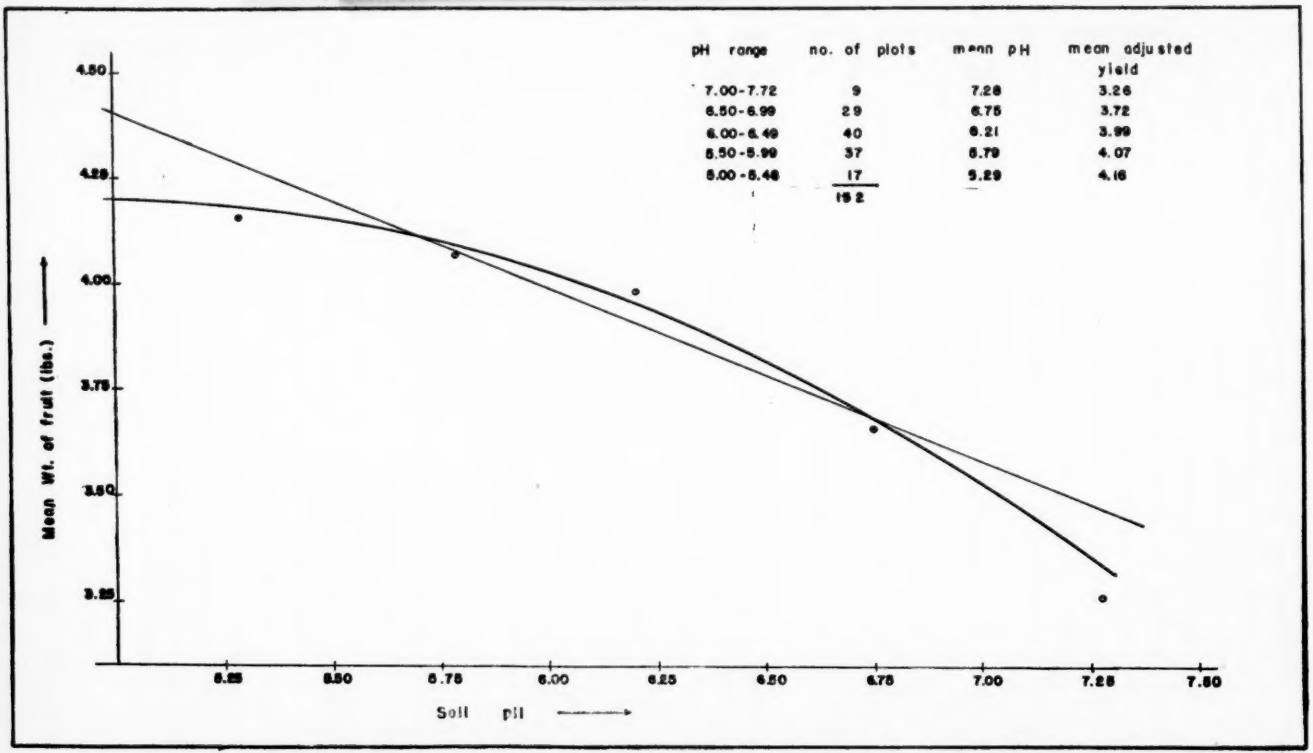

FIG. 8.—ARECIBO EXPERIMENT: Best fitting straight line and 2nd order parabold and data adjusted for calcium, nitrogen, and spray treatments and grouped with reference to pH range.

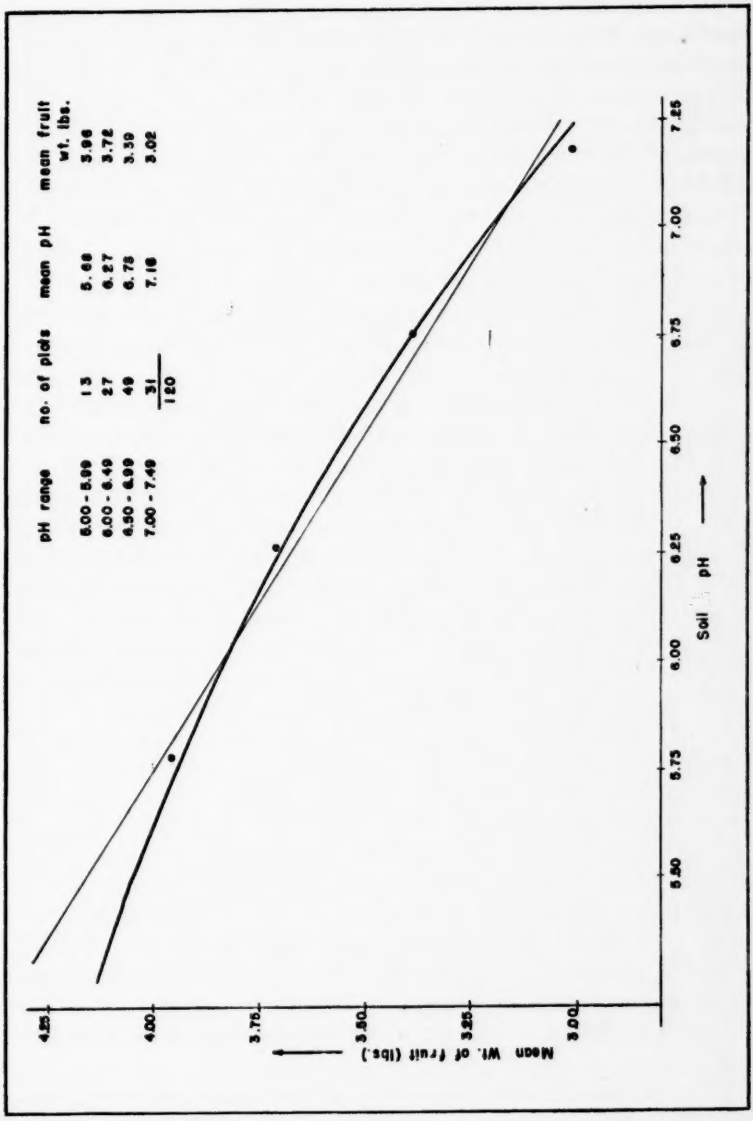

FIG. 9.—RIO PIEDRAS EXPERIMENT: Best fitting straight line and 2nd order parabola and data adjusted for calcium, nitrogen and sprays and grouped with reference to pH range.

Generalizations in terms of a straight line are best limited to the range from pH 5.70 to 6.80 where the two curves cross. Within this range there was an increase of .41 lb. per fruit for each unit decrease in mean soil pH. Beyond 5.70 further increase in acidity was attended by diminishing increment which dropped off the zero at pH 5.00. Further increase in acidity beyond 5.00 would be expected to decrease yields. The pH range in this experiment was from pH 5.02 to 7.72. The regression curve would be expected to hold true, within this range, for this same soil under similar conditions.

As may be seen in figure 9, in the Río Piedras experiment, the straight line (which is more precise than the 2nd order parabola) shows a gain in weight of .64 lb. per unit decrease in pH. This holds true within the experimental range from pH 5.21 to 7.44. However, from our knowledge of plant response to soil pH, we would not expect this straight line response to hold true in cases of extreme soil pH, outside of the experimental range. On the acid side of this range we would expect the added increment to drop off with increasing acidity as it approached toxic values. Since the parabola reaches its maximum at pH 4.27 and we know from the work of Schapelle [1] that, even in water cultures, a pH below 4.00 is injurious to pineapples, we believe that the parabola does not overstress the probable drop in increment at the acid extreme. It seems probable that if our experiment had included more plots in the acid range, between pH 4.00 and 5.00, the data would have given a significant 2nd order parabola with constants similar to the ones obtained. Since a highly significant F value was obtained for the regression curvilinear on pH the maximum at pH 4.27, with a mean fruit weight of 4.28 lbs. may be regarded as the optimum pH and maximum yield for Fajardo clay, within experimental conditions. Moreover, for forecasting probable yields on the acid side of the experimental range the second order parabola gives us more conservative estimates, which are in better agreement with our knowledge of plant response to soil pH, than does the straight line.

Using the second order parabolas in both cases, the estimated yields for both experiments are the same at pH 5.00 with a mean fruit weight of 4.20 lbs. In Arecibo the mean soil pH of unlimed plots was 5.919, while at Río Piedras it was 6.182. This indicates that the original mean soil pH, before liming, was

[1] Schapelle, N. A. Journ. of Agriculture of University of P. R., Vol. XXVI No. 3 July 1942.

fairly close to pH 6.00 in both experiments. In Arecibo, reducing the mean pH from 6.00 to 5.00 would be expected to increase yields from 4.04 to 4.20, a net increase of .16 lbs., a per acre equivalent of about 1162 lbs. or $35. In Río Piedras reducing the mean soil pH from 6.00 to 5.00 would be expected to increase yields from 3.84 to 4.30, a net increase of .36 lb., a per acre equivalent of about 2614 lbs. or $78.

Discussion:

With respect to nitrogenous fertilizer, the experiments showed that ammonium sulfate was superior to ammonium nitrate by a per acre equivalent of about $42 in Arecibo and $90 in Río Piedras. This is in agreement with the findings of many workers to the effect that ammonium nitrogen is generally a better source of nitrogen for pineapples than nitrate nitrogen.[1] As previously mentioned, this superiority of the ammonium sulfate may have been to some extent associated with a change in the soil pH in the immediate vicinity of the plant bases which was not registered because of our method of soil sampling. The difference in response to the two nitrogenous fertilizers was more than twice as large in Río Piedras as in Arecibo. The pH effect was also much greater at Río Piedras than in Arecibo and thus consistent with this suggested possibility. The matter of soil buffer action against acidification by ammonium sulfate would, of course, also have to be considered. There is no information on this score and rationalization, in view of the difference in lime requirement in the two soils, is too abstruse to carry much weight. We do not, however, see any contradiction to the above suggestion in the fact that the lime requirement was higher in Río Piedras than in Arecibo.

With regard to the effect of spray treatments, the Arecibo data showed that multiple spraying increased yields by a highly significant amount equivalent on an acre basis to $50, while in the Río Piedras data the increased yields were not significant. These results suggest that the available iron or the ratio of available iron to available manganese was lower in Arecibo than in Río Piedras.

With reference to the effect of calcium soil supplements, the experiments have shown that, apart from soil pH effects, the lime carbonate increased yields by a highly significant amount in Arecibo, but had no effect in Río Piedras. The rock phos-

[1] See Sideries C. P. Plant Physiology,—13:489–527; 1938.

phate under limed conditions had a slight, not significant depressing effect, on yields in Arecibo and a significant depressing effect in Río Piedras. The experiments did not include a non-limed rock phosphate treatment. Presumably the deleterious effects of rock phosphate are attributed to supplying added calcium over and above the calcium supplied by the lime or to the phosphate radical or to some impurity. The beneficial effect of lime carbonate in Arecibo can hardly be explained in terms of iron manganese relationships.

It seems pertinent to emphasize here that the experimental field in Arecibo had been under almost continuous pineapple cultivation during the past 15 years, whereas, the Río Piedras field had previously been in pasture and never in pineapples. It may well be that the Arecibo field may have been depleted of much of its calcium by pineapple cropping and fertilizer practice.

With reference to soil pH effects, the experimental results show that the original assumption of linear or nearly linear yield response to soil pH was warranted. The yield response was contrary to theoretical expectations based on the probable effect of soil pH on the available iron-manganese ratio and the effect of this ratio on yields. Data concerning this ratio are not yet available. It seems evident, however, that if such an improvement in the ratio was obtained its possible beneficial effect was completely masked by other factors favored by acid soil reaction within experimental limits. Our work does not discount the importance of iron-manganese nutrition of pineapples. It simply shows that raising the soil pH in an effort to improve this ratio by adjustment to slightly above pH 6, through liming, is not a promising technique for increasing yields of pineapples in the field. It could be that the yield response to pH in both experiments was due to increased availability of the iron without, for some reason, a parallel increase in available manganese. We believe, however, that factors, other than iron and manganese availability, were chiefly concerned because of the notable lack of interaction between spray treatment and the liming treatments in both experiments. Liming affected soil pH greatly in Arecibo and had an even greater effect in Río Piedras where the yield response could be explained entirely in terms of soil pH alteration.

PRACTICAL RECOMMENDATIONS

With reference to nitrogenous fertilizers, ammonium sulfate (which is a cheap source of nitrogen) should be used in preference to ammonium nitrate for pineapple fertilization, since it increased yields from $42 to $90, on a per acre dollar basis. In view of the low soil pH optima for pineapples in the two soils under experimentation there seems to be little danger in the possibility of over acidulation of the soil through prolongued use of ammonium sulfate. Such acidulation might in fact be quite advantageous.

With reference to ferrous sulfate spraying, a multiple spray schedule can under some conditions increase yields as much as $50 on a per acre dollar basis. Though our experiments did not include a multiple schedule of a few sprayings we, nevertheless, believe that our multiple spraying may have been too numerous, at least from the practical farmer's point of view. Frequent spraying has the disadvantage of increasing lesions in leaves which may result from accidental, excessive applications which drain into the heart of the plants and burn the young leaves. Moreover the eyes of the early blooming fruit are blackened when the planting is sprayed after such fruit have emerged. We believe that three to four sprayings for the plantilla cycle and about two sprayings for the ratoon would give good results with the Red-Spanish variety, under our conditions. We therefore recommend such practice tentatively, until such a time as we can experiment further along these lines. Spraying improves both the color and the size of the fruit, but fruit size response is dependent on the timeliness of the applications. We, therefore, suggest that two sprayings should be made during the first three months from planting, the second spraying to be applied after replanting. Thereafter followed by one or two spraying at 3–4 months intervals. The cost of spraying amounts to only $1.50 to $2 per acre. The farmer consequently has very little to lose and much to gain by increasing the present generally practiced schedule.

With reference to calcium carbonate soil supplements we are not yet in a position to make final recommendations. Some increase in yields, similar to those in Arecibo, are possible but losses, such as those at Río Piedras, caused by raised soil pH are also quite possible. We suggest that farmers, who want to try liming their pineapples soils, should do so very conserva-

tively and on an experimental scale. Long continued cropping of soils in pineapples, with its unbalanced fertilizer practice, may bring about deficiency of calcium in the soil. This matter is the subject of further investigations now being carried out in the field.

With reference to soil pH, the practical recommendations are obvious. Acid soils with a pH of 5.00 or slightly below are best suited to pineapples at least in the two typical pineapple soils of the experiments. This is in general agreement with the pineapple literature. There is no field evidence to support Hopkins theoretical recommendation of adjusting soil pH to 6.2. Experimentation is under way testing the possibility of increasing yields by acidulation of slightly acid, neutral and alkaline soils.

ANTHRACNOSE OF THE ANNONACEAE IN PUERTO RICO

L. A. ALVAREZ GARCÍA [1]

INTRODUCTION

Soursop or "Guanábana" (*Annona muricata L.*), Wild soursop or "Guanábana cimarrona" (*A. montana* Macf.), Wild Bullocks Heart or "Cuyar" (*A. glabra L.*), Bullock's Heart or "Corazón" (*A. reticulata* L.) and Sweestop or "Anón" (*A. squamosa* L.), are annonaceous shrubs and trees found growing in Puerto Rico in a wild state. They are more abundant on the semi-arid regions of the south and northwest on hillsides and along the coastal plain in such soils types as Descalabrado, Múcara, Guayama, Aguilita, Aguirre, San Antón, Santa Isabel, Coto, Tanamá, Camagüey, Moca and also on other similar series or types.

Under the prevalent dry environment of these regions, the *Annonaceae* are found to produce fairly good crops. However, in the humid areas of the interior and the northeastern part of the island, where there are shrubs and trees, the amount of fruit produced is commercially insignificant.

The scarcity of fruit produced in these humid areas is due to the constant drop of flowers and young fruits and the consecuence of frequent attacks of the fungus *Colletotrichum (Gloeosporium) gloeosporioides* Penz.

In Puerto Rico, strains of this organism are found associated with wither tip and fruits rots on citrus, mangos, avocados, papayas, guavas and other hosts. The organism has not been reported causing great damage to the *Annonaceae* in Puerto Rico, due perhaps to the fact that these plants have escaped the disease in their dry, natural habitat in the south coast. It has been noticed, during our field observations in the humid areas, that the organism is responsible for a constant drop of flowers and young fruits as well as for the rotting of ripened fruits.

Small (7), reports a species of *Colletotrichum* in Uganda resembling *C. coffeanum* on *Annona muricata*, though without

[1] Phytopathologist, Agricultural Experiment Station, Río Piedras, Puerto Rico.

establishing the nature of association of the organism either as a parasite or a saprophyte.

Ciferri (5), reports from the Dominican Republic the occurrence of a fruit rot of *Annona muricata* associated with a species of *Colletotrichum* (*Glomerella*) *cingulata*.

A report (1) from the Philippines mentions a species of *Colletotrichum* sp., on Atis (*Annona squamosa*).

Bensaude (3), reports *Glomerella cingulata* occuring on nearly riped annona in the Azores.

Arruda (2) found G. *cingulata* on *Annona* sp. and a canker in an *Annona* sp. of unknown origin.

Glomerella cingulata can infect banana, tomato, mango, papaya, pear, citrus, cherry, peach, olive, quince, almond, pepper and many other hosts.

In Puerto Rico the organism responsible for the wither-tip and fruit rot of the *Annonaceae* is apparently limited in its virulence by the atmospheric humidity, which seems to correlate with our field and laboratory findings. The trees growing under semi-arid conditions escape the disease, for there is little or no flower or fruit drop and the trees bear fruits until well ripe without showing any of the characteristic anthracnose fruit rots.

At present there is a strong campaign to increase fruit production in Puerto Rico. Large soursop seed beds were made during the last two years in the vicinity of Vega Baja with the purpose of planting soursop trees in waste land in that neighborhood. During the late part of the year 1944 and early in 1945, soursop seedlings in the plant beds of that community were struck by a severe epiphytotic in the form of a damping-off and die-back of soursop seedling and small trees. Many thousand, if not all the plants, were killed. Trees taken out and planted in the field succumbed to the disease and eventually the project was discontinued. A few diseased trees were taken out and planted on the south coast and in that environment they were able to overcome the disease.

It is therefore pertinent to indicate that there is the possibility of producing soursops in large scale plantings in the semi-arid regions of the south coast of Puerto Rico, where the anthracnose disease does not seem to be serious.

The results of our findings, in the field and in the laboratory, are presented here as a contribution to the understanding of some of the fruit-tree diseases in Puerto Rico.

SYMPTOMS OF THE DISEASE

Soursop plants have been found to be susceptible in all stages of development, particularly in the seedling stage. The symptoms are evidenced in the seed beds and in the field. The seed bed symptoms are first recognized by the blackening of the neck of the seedling at the time of breaking out of the soil. The lesions are dark. The infected areas enlarge and encircle the stem or involve the whole seedling.

In moist weather the organism froms dark lesions on the stems along their entire length or more commonly below the place of attachment of the cotyledons. The first lesions are brown and in some instances these cankers become deep and the stem colapses.

Some seedlings, that have escaped infection before the first true leaves are formed, drop the cotyledons and appear as if they have been decapitated. This is usually a natural proceedure in the germination and development of the soursop seedlings before they start opening the true leaves. At this stage, however, infections on the stems spreading from the tips downward were frequently observed. Usually the seedlings blighted.

On young leaves of soursop seedlings, the anthracnose appears as small, light green spots, which, under a high relative humidity, enlarge and form big patches. The lesions expand until the whole leaves are scorched and finally drop to the ground. From the leaves, the organism spreads down by way of the petioles until it reaches the yound twigs. These in turn are killed by the fungus and a particular condition known as die-back is produced. Fig. 1.

The twigs which have died-back are either abscissed by the fungus or become brittle and break down, leaving the main branches bare. The small trees look as if they have been pruned. Black cankers are produced at the point of attachment of the twigs to the stems. The cankerous lesions on the stems show characteristic splitting of the cortex and invasion of the tissues of the inner wood by the fungus. Fig. 2. These cankers resemble very much those under study by the author on citrus causing fruit and tree gummosis, which to our surprise

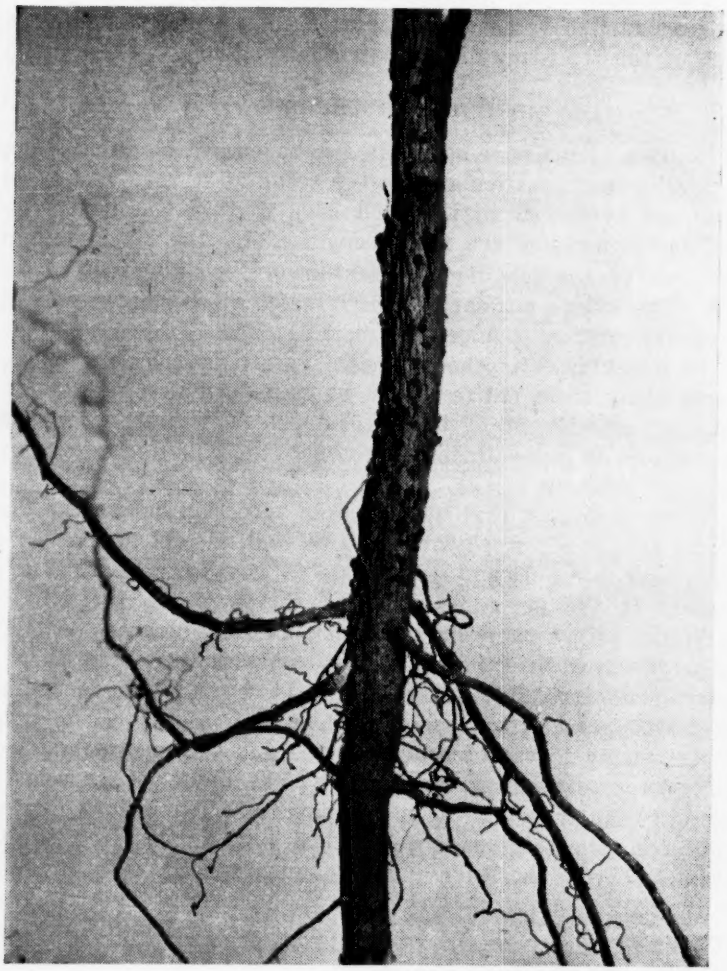

FIG. 1.—Bare effect due to the constant die-back and abscission of shoots attacked by the fungus. *Colletotrichum (Gloeosporium) gloeosporioides* **Penz.**

is caused by the same organism as that responsible for the damping-off and die-back of the *Annonaceae*. The occurrence of the citrus gummosis is reported here for the first time in Puerto Rico, and elsewhere, and will be the subject of another paper. It is interesting to indicate, however, that though the same organism is involved in these two cases, the cankers produced on the *Annonaceae* do not exhude gum, which is one of the characteristics of the citrus die-back in Puerto Rico.

FIG. 2.—Cankers on stems due to the attack of the fungus. *Colletotrichum (Gloeosporium) gloeosporioides* Penz; showing splitting of tissues.

The constant die-back of the twigs, the constant formation of new shoots from the infected stems and the re-infection and die-back of new shoots usually produce a witches-broom effect on diseased soursop trees. Fig. 3. Large trees in semi-arid regions are found almost free from die-back.

Abundant flower and fruit drop occur on trees growing inland and on the northern, humid regions of the island. Infected flowers are characterized by the appearance of dark, buff colored spots on the petals, which gradually enlarge and cover the flower. The infected flowers turn black and fall to the ground.

Young fruits when infected either rot or mummify. As soon as the organism has gained access to the peduncle, the young

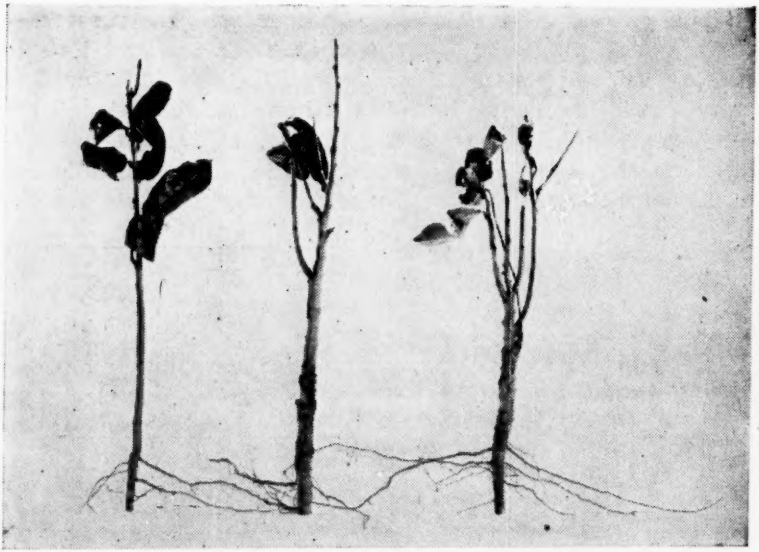

FIG. 3.—Die-back of soursop seedlings attacked by the fungus *Colletotrichum (Gloeosporium) gloeosporioides* Penz; resulting in a witches broom effect.

fruit drops to the ground. If the air and soil moisture have been high, the fruits decay rapidly. If the weather has been dry, the fruits mummify. Fig. 4.

Whenever infection occurs on matured fruits, the characteristic lesions consist of small, sometimes inconspicuous, spots on the skin of the fruits. When such fruits are cut open, the organism is found to have gained access to the juicy, acid-sweet, innermost tissues. In other instances the organism produces inside the fruit a peculiar wedge or plug structure of hardened, infected tissues without accompanying bad odors. This type of infection is somewhat of a localized dry rot. No external macroscopic symptoms of infections are detected in such types of rots.

THE CAUSAL AGENT OF INFECTION

The causal organism responsible for the flower and fruit drop, fruit rot, damping-off and die-back of soursop seedlings and trees is a polymorphic fungus known by the taxonomic name of *Colletotrichum* (*Gloeosporium*) *gloeosporioides* Penz., and commonly as the anthracnose fungus. Many strains of this and

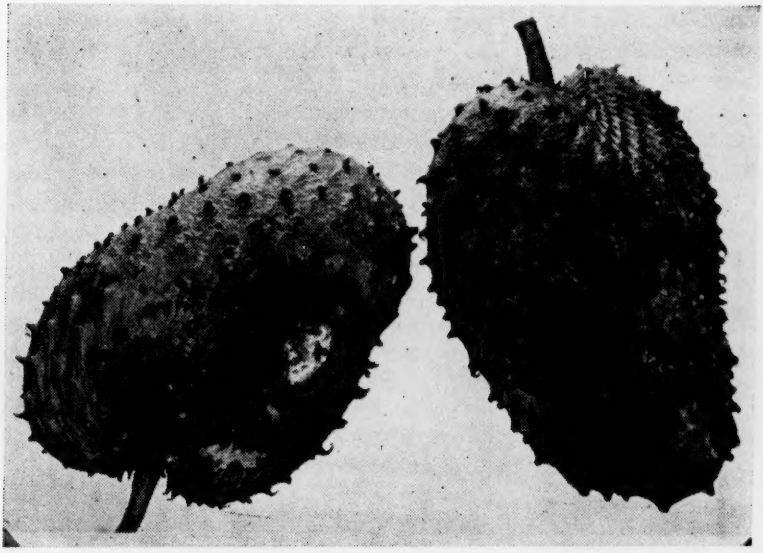

FIG. 4.—Artificially inoculated fruits, left with Form I, (b) right with Form II.

similar fungi have been reported throughout tropical and subtropical regions of the world. Some investigators have claimed that they are imperfect forms of the ascomycete, *Glomerella cingulata*.

Burger (4), studied the cultural characters of various strains obtained from citrus and separated five distinct groups, based on the type of growth and sporulation habits of the organisms.

Toro (8) separated *Gloeosporium Musarum* Cooke and Massee into eleven groups, according to cultural characters, but he did not find any significant differences as to spore measurements, even though the strains were cultured on various kinds of media.

Nolla (6) indicates in his studies on the anthracnose of citrus fruits, mango and avocado that though under artificial conditions of growth the various isolates of *G. Gloeosporioides* showed distinct cultural characters, the differences were not sufficient cause for separating them into independent strains.

The studies conducted with isolates of *G. gloeosporioides* obtained from soursop, sweet sop and bullocks heart, either from

flowers, fruits, twigs, stems or damping-off seedlings, showed when cultured in the same media, no significant differences in spore measurements (3–7 × 9–24) : indicating that in all cases, they are, perhaps, replicates of the same organism. It was also of interest to find that the spore measurements are within the limits of measurements of strains isolated from citrus, mango, coffee, papaw and other fruits reported by various workers. Penz, in his original description of *Gloeosporium gloeosporioides* gave measurements of 16–18 × 4–6u.; Burger (4), 11.5–20.3 × 3.2–64u. as an average of 100 strains.

The isolates from soursop are found to fall into two main groups, one characteristically dark-olivaceous in color, and the other definitely white when considered immediately after isolation in potato-dextrose-agar. Fig. 5 (a, b)

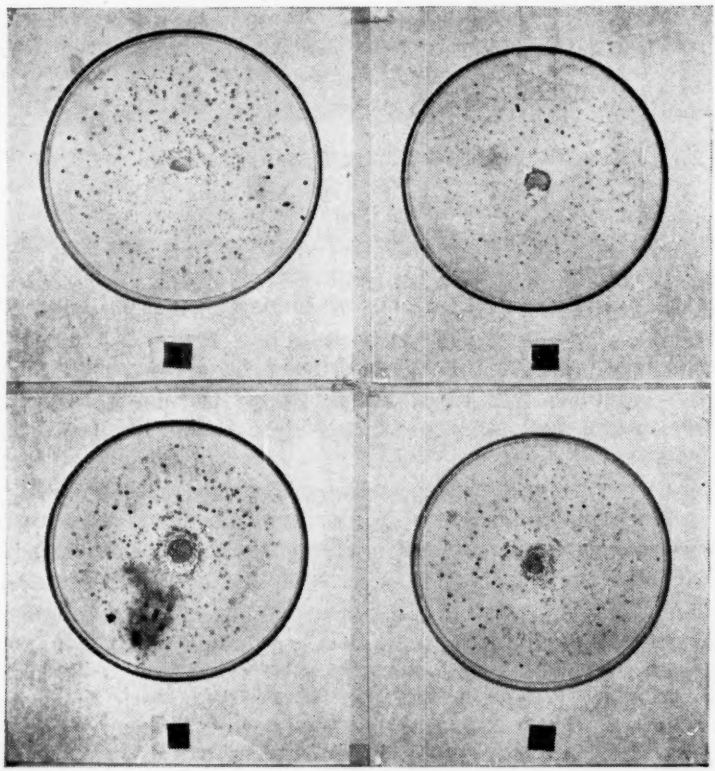

FIG. 5 (a).—Isolates from soursop. Form I.

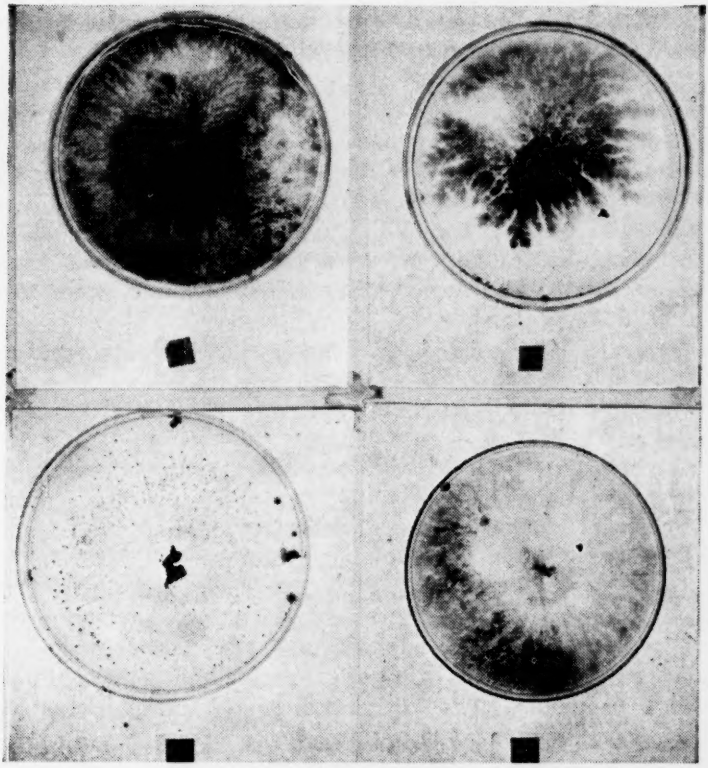

FIG. 5 (b).—Isolates from soursop. Form II.

STUDIES ON POLYMORPHYSM

In order to determine whether these forms are separate strains or are two polymorphic forms of the same fungus, transfers of the two forms were made succesively during six months, in different culture media. The results obtained after many trials demonstrated that the two forms are unstable variations of the same organism.

It is necessary to give a description of the cultural characters of the two forms at the time of isolation and then proceed to describe their behavior when grown on different culture media, to show that the two are the same fungus.

Form I, when grown on potato-dextrose-agar (pH 6.00); developed a white mycelial growth, superficial in nature, radial,

and produced pinkish or salmon color spore masses, which turn dark with age. No setae are produced. The diameter growth of the colony in seven days attained 10.5 cm.

On cornmeal agar (pH 6.00); mycelium superficial, flat, somewhat moist, hyaline or white. Sporulation occurs in 7 days, the spore masses are of an apricot-yellow color, the spores are not abundant. Spore masses turn black in old cultures, and no setae are produced. Growth of colony in seven days is 8.75cm.

On nutrient agar (pH 6.8); mycelium white, superficial or slightly trophic, radial, somewhat fluffy at border of colony. Spore masses, pale yellow, scattered. Colony growth in seven days, 5.25cm. in diameter.

On prune agar (pH 5.6) mycelium whitish, scanty, thin and growing flat on the surface of medium, mycelium turns olive green. Sporulation poor or not at all. Growth of colony in seven days is 8.50cm.

On oatmeal agar (pH6.5); mycelium scanty, growing flat, white except for some small sectors near the center. Sporulation poor. Diameter growth of colony in seven days 8.50cm.

FORM II:

On potato-dextrose-agar (pH 6.00); growth fluffy, somewhat aerial, white in young colony, turning gradually grayish to olivaceous with age and forming tufts or clumps of thick-walled mycelial threads; growth of mycelium somewhat arborescent on surface of a substratum tinged dark. Chlamydospores very abundant, thich-walled, granular or filled with oil globules, sporulation infrequent and very poor. Spore masses small, dark, seldon pinish, or yellowish. Growth of colony in seven days 9.00 cm. Spores granular or oily.

On bean-dextrose-agar (pH 5.6); superficial growth of mycelium, white when the colony was young, turning dark when old. Sporulation poor. Diameter growth in five days 8.50cm.

On cornmeal-dextrose-agar (pH 6.00); mycelium flat, or somewhat aerial, wooly, olive green, dendriform, radial. Growth rapid attaining 9cm. in five days. Sporulation poor.

On Ashby synthetic medium (pH 7.0); mycelium at first white, scanty, very poor growth, turning dark. Sporulation poor, spore masses black, chlamydospores abundant.

It is interesting to notice that in the first trials with Forms I and II; Form I always formed white mycelium and colored spores masses and Form II produced dark mycelium and sporulated poorly or not at all. However, with continued transfering,

Form I and Form II showed great variability in their morphological character; Form I produce dark sectors and Form II, white or grayish sectors. Form I monosporial cultures from completely white, (Form I) colonies when sub-cultured in different media produced colonies like Form II. Monosporial cultures obtained from completely dark (Form II) colonies produced white types colonies like Form I. Fig. 6.

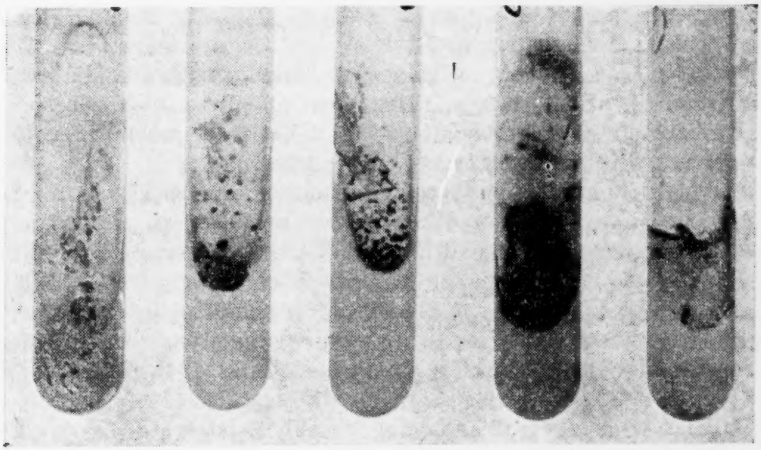

FIG. 6 (a)

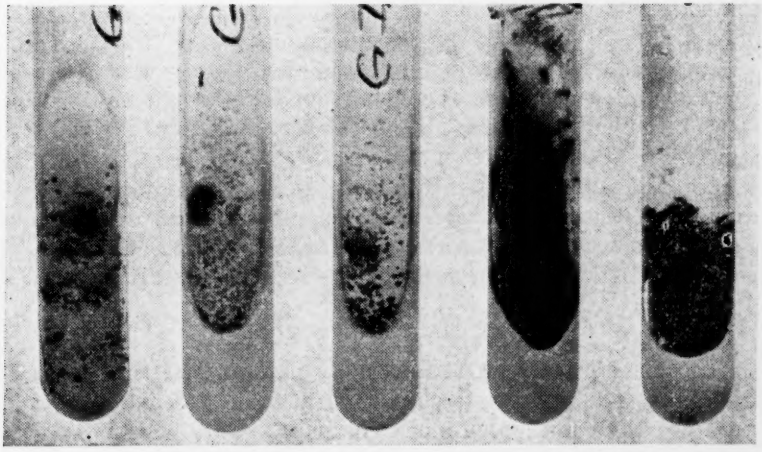

FIG. 6 (b).—Monosporial cultures obtained (a) Form I, and (b) Form II, showing their similarities when grown on potato-dextrose-agar adjusted to varying pH of the substrate.

Hyphal-tip transfers of white sectors or variants stayed white, but upon subsequent hyphal-tip transfers from this culture, grayish or black and white colonies were produced. The reverse picture appeared when hyphal-tip transfers were made from white or grayish sectors of Form II.

Dark colony types obtained by subsequent transfers from either Form I or II, or from white or light colored sectors of Forms I or II, sporulate abundantly. In high proteinous media the spore masses are pink or salmon colored. Formation of thick-walled chlamydospores takes place readily in dark colonies. Form I generally produces thin-walled clamydospores and abundant spores. Camera lucida drawings of spores of the respective variants of polymorphic forms do not show much difference in shape, size and appearance of the protoplasm.

Polymorphism is apparently associated with the type of culture media and perhaps other concominant, environmental factors which could not be studied at the time because of the lack of adequate equipment for such type of work. Our purpose has been to (1) study the occurrence of the disease under varying ecologic factors, (2) to understand its prevalence in some areas and (3) to determine possible methods of control.

Small (7) on a discussion on the occurrence of species of *Colletotrichum* indicates that the term *C. coffeanum* could be used to designate, with equal or greater justification, the various conidial forms of this fungus except for *C. gosypii* and *C. lindemuthianum,* whose ascigerous, *Glomerella* stage has never been reported.

In Puerto Rico all conidial forms of *Colletotrichum* (*Gloeosporium*) *gloeosporioides* could be put together in one group; if we consider that there are no significant differences in form and size of spores and that the strains, as has been shown, can induce similar, typical anthracnose lesions on different hosts upon cross-inoculation. It is pertinent to point out that in Puerto Rico the perfect form *Glomerella cingulata* has not been reproduced in culture or reported as occuring in nature.

MATERIALS AND METHODS

Inoculation tests on anthracnose, damping-off and die-back of soursop seedlings were conducted inside a greenhouse and in an especially constructed moist chamber. Data on air temperature and relative humidity were obtained with a Friez recording hygrothermograph.

The soursop seedlings utilized during the course of the experiments were grown from fresh, non-infected seed sown in sterile, steamed soil in flats.

Seeds were sown in July 1944 and were used for inoculation as soon as their first true leaves had developed. Subculture of *Gloeosporium gloeosporioides*, Forms I and II, were used throughout the work, in parallel tests.

Artificial inoculation of soursop seedlings were conducted during November and December, 1945 and February and March 1946 at the time when the seeds were germinating, about four to six weeks after planting. The seedlings were sprayed with a spore suspension using a De Villbiss atomizer. The sprayed seedlings were placed and kept inside moist chamber for three weeks. Flats with seedlings were inoculated in the same manner and were placed inside a greenhouse. Seedlings grown in flats, as controls, were similarly treated, but this time they were sprayed with sterile water. Some of the controls were placed inside a moist chamber and others inside the greenhouse.

The results of these trials, three to four weeks after inoculation, showed the susceptibility of soursop seedling to infection with the fungus *Gloeosporium gloesporioides* (Form I or II).

The seedlings kept inside moist chambers (80 per cent relative humidity) showed the characteristic damping-off and blight symptoms at the end of four weeks.

Inoculated seedlings placed inside the greenhouse (40 per cent relative humidity) did not become infected and continued developing free from anthracnose lesions. The controls kept in either moist or dry environments were healthy at all times.

TABLE NO. 1—Susceptibility of recently emerged soursop seedlings (with either one or two pairs of leaves opened or in the cotyledonous stage) to infection with *Gloesoporium gloesporoides* Seedlings sprayed with spore suspension of the organism were placed in a moist (80% relative humidity) chamber for four to six weeks or inside a greenhouse with a 40% relative humidity.

Inoculum	Environment: Relative Humidity	Cotyledonous Seedlings Inoculated	Seedlings with One to Three Pairs of Leaves	Cotyledonous Seedlings Diseased	Seedlings with One to Three Pairs of Leaves Diseased
	per cent	number	number	number	number
Form I	40	40	40	0	0
Check	40	20	20	0	0
Form II	40	40	40	0	0
Check	40	20	20	0	0
Form I	80	40	40	32	30
Check	80	20	20	5	2*
Form II	80	40	40	39	37
Check	80	20	20	10	3*

* Infections were probably due to spores floating inside inoculating chamber after inoculating seedlings. No infection occured in seedlings kept as checks under bell jars under similar conditions of high relative humidity.

Six or eight months old seedlings, inoculated and treated as explained previously, showed evidence of anthracnose lesions one to two weeks after being inoculated. The anthracnose and die-back symptoms were evident two to three months after inoculation. Cankers were formed at the end of four months at the point of attachment of the twigs to the main branches or stems.

The cankers on stem and branches were characterized by a cracked cortex and blackened inner wood. There was no gum formation in the cankers. Die-back of new shoots attacked by the fungus was continual and a peculiar witches-broom effect developed.

TABLE No. 2—Influence of relative humidity upon the occurrence of anthracnose on six-months old soursop, *Annona Muricata*, caused by *Gloeosporium gloeosporioides*. Greenhouse temperatures from 22° C. at night to 32° C. during daytime.

Inoculum	Source	Relative humidity	Plants inoculated	Blighted plants	Blighted plants
		per cent	number	number	per cent
G. gloeosporioides F-I	Soursop	40-80	10	9	90
G. gloeosporioides		40-50	10	0	0
G. gloeosporioides F-II	Soursop	40-80	10	10	100
G. gloeosporioides		40-50	10	0	0
G. gloeosporioides F-I	Sweetsop	40-80	10	9	90
G. gloeosporioides		40-50	10	0	0
G. gloeosporioides F-II	Sweetsop	40-80	10	10	100
G. gloeosporioides		40-50	10	0	0
G. gloeosporioides F-I	Bullock's Heart	40-80	10	10	100
G. gloeosporioides		40-50	10	2	20
G. gloeosporioides F-II	Bullock's Heart	40-80	10	10	100
G. gloeosporioides		40-50	10	1	10
G. gloeosporioides F-I	Citron	40-80	10	9	90
G. gloeosporioides		40-50	10	0	0
G. gloeosporioides F-II	Citron	40-80	10	10	100
G. gloeosporioides		40-50	10	0	0
G. gloeosporioides F-I	Papaw	40-80	10	10	100
G. gloeosporioides		40-50	10	0	0
G. gloeosporioides F-II	Papaw	40-80	10	10	100
G. gloeosporioides		40-50	10	0	0
Control		40-80	10	2	20
Control		40-50	10	0	0

Soursop seedlings kept inside a greenhouse showed few leaf infections with *G. gloeosporioides* but they never enlarged to form blotches or involved the entire leaf.

The anthracnose leaf spots became restricted in their development under a relative humidity not beyond 50%.

Infections were not possible in an environment with a 50 per cent relative humidity, or less. Infected plants when taken out from a wet chamber and placed in an atmosphere of 50 per cent relative humidity recovered from the malady and produced healthy shoots.

Several experiments were conducted during February and March 1947 to determine the effectiveness of several fungicidal sprays in controlling anthracnose.

Three months old seedlings were potted and placed in a dry atmosphere of 40 per cent to 50 per cent relative humidity and after recovering from the shock of transplanting the plants

were moved to a 60 to 80 per cent relatively humid environment in a moist chamber. After three weeks no symptoms of antracnose and die-back were evidenced; thus indicating that natural infections have not occured.

The seedlings were divided in lots of 10 pots each and kept at 60 to 80 per cent relative humidity and a temperature from 22 to 30°C. Each lot was sprayed with Bordeaux (4–4–50); Phygon (1 lb. to 100 gal.); Zerlate (2 lb. to 100 gal.) and Fermate (2 lbs. to 100 gal.), respectively. The fungicides were allowed to dry on the seedlings. The seedlings were then sprayed with a spore suspension of *Gloeosporium gloesporioides.* Control plants were sprayed with sterile water then inoculated and placed under bell jars.

Two weeks later the plants were similarly treated with fungicides and re-inoculated. The results of the test are summarized in table 3.

TABLE NO. 3—Test of fungicides applied as sprays for contro of anthracnose of soursop (*Annona Muricata L.*). Seedlings artificially inoculated with a spore suspension of the fungus *Gloeosporium gloeosporioides* Form II, the same day after they were sprayed with the fungicides. Sprayed plants and non-sprayed checks were placed inside a moist chamber (80% relative humidity) for four weeks.

Fungicides	Sprayed Seedlings Inoculated		Non-sprayed Seedlings Inoculated		Blighted Seedlings	Spots per leaf	Blighted Seedlings	Leaf spot per plant
	Seedlings	Total leaves	Seedlings	Total leaves				
	number	number	number	number	number	average	per cent	per cent
Zerlate	10	45	10	40	0	1	0	10
Fermate	10	42	10	41	1	1	10	10
Bordeaux	10	40	10	43	3	5	30	50
Phygon	10	43	10	40	2	3	20	30
Check*	10	44	10	42	10	6	100	60

* Sprayed with sterile water, then inoculated with a spore suspension of the pathogen.

Damping-off, seedling blight, die-back and canker formation were reproduced by artificial inoculations with either Form I or, II of *G. gloeosporioides.* The pathogen was invariably reisolated from the infected or diseased seedlings.

DISCUSSION AND SUMMARY

The incidence of *Gloeosporium gloeosporioides* anthracnose on the *Annonaceae* in Puerto Rico seems to be correlated with high relative humidities. Experiments have shown that the *Annonaceae* are seriously attacked by the anthracnose fungus in the northeastern and wet inland regions of Puerto Rico. The disease is responsible for a constant drop of flowers and fruits, a die-back of twigs and branches and a damping-off of the seedlings.

The growing of *Annonaceae* is commercially impossible under unfavorable environmental conditions, such as high atmospheric moisture. *Annonaceae* are found growing almost free from anthracnose in the dry south and southwestern part of Puerto Rico, developing well and producing heavy crops.

The casual agent of infection is a polymorphic organism, involving many forms, exhibiting different morphological characters, such as color of colony, color of spore masses, and type of mycelium.

These forms are biotypes and caused typical anthracnose of the various *Annonaceae*.

The greenhouse experiments revealed that infection can develop promptly on inoculated plants placed inside moist chambers with 60 to 80 per cent relative humidity within three to four weeks. The typical symptoms observed in the field were reproduced upon artificial inoculation.

No incidence of anthracnose was seen on inoculated plants kept at all times inside a greenhouse with a 40 to 50 percent relative humidity.

The diseased plants kept inside a moist chamber, when removed and placed in a dry atmosphere of 40 to 50 percent relative humidity, recovered from the disease. The correlation between high humidity and occurrence of the disease was evident.

Fungicidal sprays were tested during 1946–47 for efficacy in controlling the disease. Plants were sprayed separately with Zerlate (2–100) Fermate (2–100) Phygon (1–100) and Bordeaux (4–4–50). After the sprays have dried on the leaves, the plants were inoculated with a spore suspension of the pathogen. Sprayed-inoculated plants were then kept in a favorable environment for infection of 60 to 80 per cent relative humidity for four weeks. The results obtained indicate that weekly sprayings with Zerlate, Fermate, Phygon and Bordeaux were effective in controlling the disease. It may be concluded that *Annonaceae* can be grown commercially in the dry southern areas of Puerto Rico without spraying. In case of extreme and unusual moist weather conditions, spraying with Zerlate or Fermate alone or in combination with Bordeaux or Phygon keeps the disease effectively under control.

Under no circumstances should the *Annonaceae* be grown commercially in a humid climate, such as is found in the northern, eastern and central areas of Puerto Rico.

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ASPERGILLUS ROOT-STALK ROT OF SANSEVIERIA
(*SANSEVIERIA LAURENTII*, WILDEM)

L. A. ALVAREZ GARCÍA [1]
and
MIGUEL A. DÍAZ [2]

INTRODUCTION

The ocurrence in Puerto Rico of a serious root-stalk disease of *Sansevieria Laurentii*, Wildem, a bud variation of the common species *S. Zeylanica* Wildd. (Bowstring hemp), has been found to hamper the exportation of slips to the United States market.

The disease hindered the exportation of this crop to such an extent, that Puerto Rican nurserymen requested technical help from the Agricultural Experiment Station and from the Agricultural Extension Service of the University of Puerto Rico.

The outcome of the technical findings is presented here as a contribution to the knowledge of ornamental plant diseases in Puerto Rico and their control.

ECONOMIC IMPORTANCE

Sansevieria plants are highly esteemed as house plants. They are characterized by a beautiful yellow band bordering the edge of the leaf blade. It is claimed that the plants are very hardy and well adapted to a great variety of environmental conditions, thriving even in extreme dry or wet conditions and under low or high light intensity.

During the early part of this century, the exportation of Sansevierias to the United States market was of relatively economic importance. The year 1920 was a boom year, but a gradual decline was then felt until 1930, when a new fad for Sansevierias revived the local industry. Then in 1936, a marked drop in exportations was felt, due to a preference for desert plants, principally from California. The local nurserymen looked for new markets and exported their crop to England, France and Belgium.

[1] Plant Pathologist, Agricultural Experiment Station, Río Piedras, P. R.
[2] Assistant Horticulturist, Agricultural Extension Service, Río Piedras, P. R.

During the Second World War exportation of Sansevierias to the United States received great attention. At present there is a great demand for the crop.

SYMPTOMS OF THE DISEASE

Aspergillus root-stalk rot, following spontaneous or artificial infection of wounded tissues of slips of Sansevierias, is characterized by a rapid invasion of the succulent tissues of the root-stalk and leaf petioles. The slips are obtained usually by cutting, but sometimes by "snapping" the brittle stems. The slips which are snapped off are known as "knock-off" slips.

Injured infected tissues become water-soaked and permeated by the profuse mycelial growth of the causative fungus. Once the parasite has gained a foothold, its growth and invasion of the host tissues is very rapid. The epidermal tissues of the root-stalk usually keep their firmness even in cases of advanced rotting of the inner tissues.

In a warm, humid environment, the percentage of diseased slips is very great, and the amount of infections is very high. In such an environment the parasite works rapidly, causing a complete rot of the root-stalk in a few days. This correlates with the behavior of the parasite when grown in artificial substrates in a warm, humid environment.

Rotting of the root-stalk advances a long way up the stalks, the leaf petioles are invaded, and the result is the abscission of the diseased leaves. Infected tissues of Sansevieria plants appear moldy and covered with a profuse superficial mycelium, which spreads readily developing masses of black spores.

THE CAUSAL AGENT

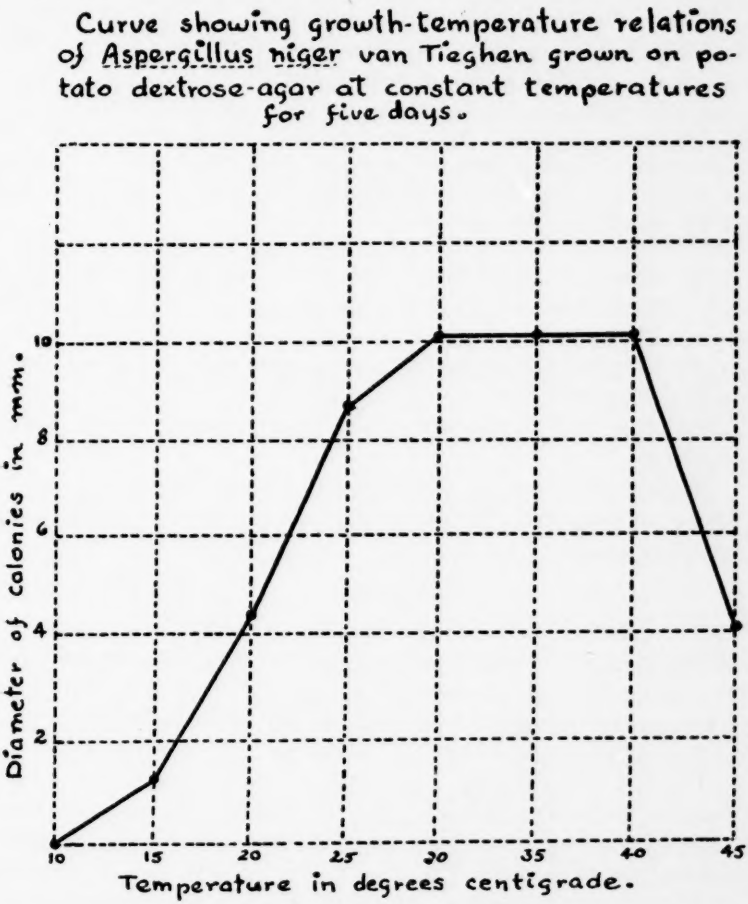
The black-mould fungus responsible for the root-stalk rot and leaf of Sansevierias belongs to the *Aspergillaceae*. Microscopic examination revealed it to be *Aspergillus niger* van Tieghem.

In Puerto Rico the senior author has found *A. niger* causing decay of garlic (*Allium sativum*) in storage. *A. niger* has been reported from Brazil, by Viegas,[10] causing bulb rot of onions (*Allium cepa* L.). *A. niger* has also been reported causing fruit decay of apples, cotton bolls, corn, and potato tubers.[6, 8, 9, 12]

The *A. niger*, responsible for the root-stalk of Sansevierias in Puerto Rico, is favorably influenced under high humidity and high temperatures, to parasitize wounded, succulent tissues of

these plants. The fungus grows readily in darkness and in few days covers the surface on most artificial media in Petri dishes.

On potato-dextrose-agar, with a pH value of 6.15, *A. niger* covered completely the 9 cm. plate in 4 days, when incubated in a constant temperature range from 25 to 40°C. No growth was obtained at temperatures below 10°C., or above 45°C.



Sporulation was very profuse at all temperatures from 25 to 40°C. The organism was found to grow within a very wide pH range, from pH 2 to pH 8. No efforts were made to determine the limits of growth on the acid or alkaline side of the range. Growth was rapid in media with a high water content.

EPIDEMIOLOGY

Slips of Sansevierias, as prepared for shipment, bear from 3 to 5 leaves, and have an approximate perpendicular length of 10 to 12 inches. When the slips are cut or snapped off from the stock plant, a profuse bleeding from the wounded root-stalk occurs.

Parasitism by *A. niger* seems to occur only through wounds on the root-stalk or leaf petioles, as confirmed by artificial inoculation of wounded and unwounded tissues.

Aspergillus root-stalk rot appeared in slips shipped during the summer and winter months. A warm environment with insufficient ventilation keeps the slips warm and moist. These are important factors associated with the serious damages reported on shipped Sansevierias.

The material for winter shipment was prepared as follows: (1) Pairs of slips were wrapped in newspaper. (2) Ten such pairs were wrapped in newspaper to form a bundle. (3) The bundles were placed in wooden boxes lined with newspaper. After these operations the bundles were ready for shipment.

Shipments of Sansevierias took about 10 days to reach the mainland.

During the summer, the slips are not wrapped in so much paper, nevertheless, great damages are reported in transit, due to root-stalk rot.

CONTROL

The remarkable performance of a number of recently developed synthetic organic compounds for the prevention of plant diseases, suggested the testing of some of them in an attempt to find an effective and practical control measure for the prevention of the aspergillus root-stalk rot of Sansevierias.

The following organic and inorganic compounds were tested: Spergon, Semesan, Semesan Jr., Dubarry 1155, Arasan, Zinc oxide, cuprous oxide, copper carbonate, mercuric bichloride, boric acid and paraffin.

TECNIQUE FOR MEASURING EFFECTIVENESS

The fungicidal value of the organic and organic compounds under consideration was determined on the basis of the number of diseased slips treated and on the number and the degree of invasion and rotting of the infected tissues of the root-stalks.

Preliminary work consisted in the treatment of lots of ten pairs of lips, immediately after being cut off from the stock plants, with the fungicidal dusts, or immersed in the solutions. The wounded tissues of the root-stalks were dressed or immersed in the solutions, as the case demanded.

The slips were treated and wrapped for shipment and packed in wooden boxes as if they were to be exported. The treated and packed slips were stored in a dark room and left for 10 days in order to observe the effectiveness of the treatments in conditions similar to those liable to occur in shipments.

The results observed in the experiments conducted during the month of December 1945 and January 1946 are summarized below:

DIPPING

Mercuric bichloride: Slips were inmersed in a 1–1000 bichloride solution for 3 minutes and immediately afterward were washed thoroughly with water, so as to remove the residual salt. It proved very effective. This treatment, however, had the disadvantage of being laborious and expensive. Moreover, there was no objective way of detecting treated from untreated slips, which is a handicap if we consider that the worker can make mistakes easily.

Mercuric bichloride without water washing: The previous technique was followed but not water washings were performed. The results were discouraging. Probably because residual salt injured the tissues, which eventually rotted.

Benzoic acid solution (3 percent in 30 percent alcohol): Tissue injury and rapid decay occured when Sansevieria slips were treated with this solution.

Paraffin (50 to 60°): This treatment was ineffective and expensive.

DUSTING

Spergon:

This treatment proved very effective by checking the disease completely. The ease in operation and the yellow color of the fungicide on treated slips made it most desirable.

Semesan Jr.:

It was very effective, however there was no significant difference in its effectiveness when compared with Spergon, or the bichloride—washing treatment.

Semesan:
Fairly good. It controlled the disease up to 50 percent.

Zinc Oxide:
Ineffective.

Corona Coppercarb:
Ineffective.

Cuprous Oxide:
Ineffective.

Results of these treatments are presented in Table 1.

TABLE No 1—TESTING FUNGICIDAL CHEMICALS FOR CONTROL OF SANSEVIERIA (*Sansevieria Laurentii* WILDEM), ASPERGILLUS ROOT-STALK ROT

Slips kept after treatment in a dark room at 30° C. for 10 days.

No.	Trade Name	Treatment	Percent of Effectiveness
1.....	Corrosive Sublimate.....	Mercuric bicholride with thorough washings.....	100
2.....	Spergon.....	Tetrachloro-benze-quinone.....	100
3.....	Semesan Jr.....	Ethyl-mercury-phosphate 1%.....	100
4.....	Semesan.....	Hydroxy-mercury-chlorophenol 30%.....	50
5.....	Zinc Oxide.....	Zinc oxide.....	10
6.....	Cuproxide.....	Cuprous oxide.....	10
7.....	Paraffin.....	Paraffin.....	10
8.....	Corona Coppercarb.....	Copper carbonate.....	10
9.....	Corrosive Sublimate.....	Mercuric chloride.....	0
10.....	Benzoic Acid.....	Benzoic acid 3% in 30% alcohol.....	0

The data in Table 1 show the effectiveness of Spergon, Semesan Jr., and mercuric bichloride with water washings in controlling aspergillus root-stalk rot.

The non-phytotoxicity and the bright yellow color of Spergon, which made recognition of the treated slips easy, and its good adhesiveness were considered in selecting it for the final shipping test.

On January 3rd and 4th, 1946, twenty-thousand slips were treated with Spergon, then they were prepared for shipment, as previously explained, and sent to New York in two lots of ten-thousand each. The report of their arrival free from aspergillus root-stalk rot, demonstrated the effectiveness of the Spergon treatment. Untreated checks also shipped, and those kept for 10 days in storage in Puerto Rico, rotted badly.

CONCLUSION AND SUMMARY

The results of the experiments conducted at Mr. Charles Pennok's farm in Río Piedras, (North and South nurseries) during the two-month period from December 1945 to January

1946, to find an effective commercial control for aspergillus root-stalk rot of Sansevieria, revealed the practical fungicidal value of Spergon, Semesan Jr. and bichloride of mercury; the latter followed by thorough washings.

Dusting with Spergon and Semesan Jr. is easy and inexpensive. Spergon, which is easily recognized in treated cuttings by its yellow color, was finally selected for the commercial treatment of Sansevierias slips prior to shipping.

This treatment of root-stalk cuttings also lessens considerably decay in seed beds. It can be used either in powder form or in water suspension.

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